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THE NUMBER OF SUBUNITS IN THE MOLECULE OF HORSE HEMOGLOBIN[1]

BY M. E. REICHMANN[2] AND J. ROSS COLVIN

ABSTRACT

The molecular weights of horse hemoglobin, horse globin, and performic acid oxidized horse globin were determined by osmotic pressure, by an approach to equilibrium sedimentation, and by light scattering (except hemoglobin) at pH 1.5 to 2.5 in 0.05 *M* NaCl. Sedimentation coefficients were determined for these materials over the same pH range and electrophoretic analyses were made from pH 1.5 to 4.0. The results show that in dilute salt solutions below pH 2.5 horse hemoglobin dissociates to four subunits all approximately equal in mass but at least two of which differ electrokinetically and therefore in composition. The subunits are probably held together in the native hemoglobin molecule only by non-covalent bonds.

INTRODUCTION

The effect of changes in pH of the medium upon the size, shape, and dissociation of a number of proteins has contributed to knowledge of their structure (11). In particular, the properties of mammalian hemoglobins in dilute acid solutions (13), in surface films (14), in solutions of urea (34), and in concentrated salt solutions (15) have indicated that the molecules may split into halves or smaller subunits. These observations, coupled with the presence of four hemes per molecule, have led to the plausible conclusion that there are four subunits in each hemoglobin (17), but definitive evidence has been lacking. In addition, Porter and Sanger's (27) observation of six N-terminal valine residues in horse hemoglobin suggests that, in some species, the subunits may be further dissociated to individual polypeptides or that branching of one or two chains occurs. To test previous conclusions, and to distinguish between the above possibilities, the molecular weights of horse hemoglobin, horse globin, and performic acid oxidized horse globin have therefore been determined by light scattering, osmotic pressure, and Archibald's approach to equilibrium sedimentation (5), at higher hydrogen ion concentrations than reported hitherto. The results were supplemented by velocity sedimentation and electrophoretic analyses.

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MATERIALS AND METHODS

Crystalline horse hemoglobin was prepared by the method of Heidelberger (19). The filter cake of twice recrystallized oxyhemoglobin was stored frozen until used. All the filter cake dissolved in water at 0°C. except a small fraction of white flocculent material which was removed by centrifugation before preparation of the globin. Two determinations of the sedimentation coefficient of the sample, $S_{w,20}$, in 0.05 *M* phosphate, pH 6.8, at concentrations of 0.25% and 0.50%, gave 4.40×10^{-13} and 4.54×10^{-13} respectively. These values are in agreement with previous results (37). An electrophoretic analysis of a 0.2% solution of the hemoglobin in 0.05 *M* phosphate at pH 6.8, time 141 min., gave a single, slowly migrating peak for both ascending and descending boundaries. The molecular weight of the hemoglobin by osmotic pressure in 0.1 *M* NaCl, pH 7.0, was 65,000.

Globin was prepared by Anson and Mirsky's method (4) as modified from Jope, Jope, and O'Brien (22). Crystalline horse oxyhemoglobin was dissolved in ice water to give a 5% solution and the supernatant poured, with rapid stirring, into 10 times its volume of 0.7% HCl–acetone at −15°C. The precipitate was removed by filtration at −15°C. and most of the heme washed out at that temperature by HCl–acetone, followed by chilled acetone. The precipitate was redissolved in ice water, reprecipitated and washed again, as above, three times. After the final washing with acetone, the precipitate was dissolved in ice water, filtered through coarse filter paper, and freeze dried. The product, which was always a light tan in color, was initially soluble in water and 0.05 *M* NaCl but reprecipitated quickly if the pH was between 4.0 and 10.0. Outside this range, solutions of globin, 1% or lower in concentration, were stable indefinitely in 0.05 *M* NaCl. Increasing the salt concentration decreased solubility at all pH values. Native horse globin, in the sense that all the product was soluble indefinitely close to neutrality, was not obtained, in agreement with previous observations of the particular instability of horse globin (31).

A modification of Sanger's method (33) was used to oxidize any disulphide bridges in globin and thus promote separation of the polypeptide chains. Each gram of unoxidized globin was dissolved in 36 ml. of 90% formic acid to which 6 ml. of 30% H_2O_2 had been added one hour before. The mixture was allowed to stand at room temperature for 25 min. (one sample was over-oxidized by extending the time to 60 min.) and then poured into 10 times its volume of 0.7% HCl–acetone at −15°C. The precipitate was removed by rapid centrifugation at room temperature, immediately dissolved in water, and dialyzed against water for 48 hr. at 5°C., with frequent changes of the external solution. The resulting optically clear solution was then centrifuged and freeze dried. The solubility properties of the white product were similar to those of unoxidized globin except that the oxidized material was initially more readily soluble in water or dilute buffer solutions but also reprecipitated more quickly. Control experiments established that less than 2% of the initial nitrogen was lost during oxidation of the globin.

Only valine was detected as an N-terminal amino acid residue in both the

unoxidized and oxidized globin by the DNFB technique (26). Since Porter and Sanger had previously found only valine as an N-terminal residue in horse hemoglobin (27) this observation is evidence that no appreciable degradation of the polypeptide chains occurred during the oxidation procedure. Other investigations have also shown that the peptide bond is resistant to attack by performic acid (3).

Both unoxidized and oxidized samples of globin were prepared for examination by dissolving the sample in water or the appropriate buffer, followed by dialysis against 10 times the volume of the buffer changed frequently.

All buffers were prepared from reagent grade materials by Clark's procedure (9) and checked frequently against commercial standards with a Beckman Model G pH meter. Where necessary, the pH of solutions of sodium chloride was adjusted by the addition of small amounts of HCl.

Crystallized bovine plasma albumin and crystallized bovine pancreas ribonuclease were used as purchased from Armour and Company, Chicago.

The techniques of molecular weight determination by light scattering were as previously described by one of us (28). All measurements were made at a wavelength of 4360Å. A monochromatic filter was also inserted in the phototube mouthpiece to eliminate fluorescent light. The refractive index increment, dn/dc, of unoxidized globin was measured in the Brice and Speiser differential refractometer (7) and found to be 0.200 ± 0.003 in 0.2 *M* NaCl, pH 2.5. With this value of dn/dc, K (28) had the value of 6.46×10^{-7}. The same figure was used for oxidized globin.

Osmotic pressures were determined by the equilibrium method. The osmometers as well as the technique used were as previously described (29). Determinations of the molecular weight of bovine plasma albumin in 0.1 *M* NaCl, pH 6.5, gave 68,000 in agreement with previously reported results (16). Prior adjustment of the osmotic pressure to close to the estimated final equilibrium value, which was then maintained for 12 hr. or more, showed the membranes to be impermeable to any component of the globin or hemoglobin preparations. The same technique showed that the membranes were readily permeable to ribonuclease, molecular weight 13,000.

Sedimentation coefficients were determined in a Spinco Model E ultracentrifuge at 250,000 *g* using a plastic cell to contain the more acid solutions. Boundary displacements were read directly from the photographic plates by a microcomparator accurate to ± 0.001 mm., but this overestimates the precision since rapid diffusion of the peak made the exact position of the mode uncertain. In calculating $S_{w,20}$, a 1° correction for adiabatic cooling of the rotor (6, 36) was applied as well as the usual adjustments for temperature and viscosity of the buffer (2). Three determinations of the $S_{w,20}$ of bovine plasma albumin in 0.05 *M* phosphate buffer, 0.2 *M* NaCl, pH 6.6, at concentrations 0.33%, 0.50%, and 1.0% gave the values 4.10, 4.08, and 3.91×10^{-13} respectively in agreement with previous results (24).

Molecular weights were determined by an approach to equilibrium sedimentation using Archibald's method (5) as recently applied by Brown, Kritchevsky, and Davies (8). All measurements were made at a rotor speed of 12,590 r.p.m.

The molecular weight of globin was calculated from the extrapolated values of $(1/rc)(dc/dr) = \delta = M\omega^2(1-V\rho)/RT$ (M = weight average molecular weight, ω = angular velocity, V = partial specific volume of protein, ρ = density of solution, R = gas constant, and T is the temperature) using the partial specific volume, 0.749, as reported for hemoglobin (37).

Concentrations of protein were estimated by micro-Kjeldahl, using Tristram's value for the nitrogen content of hemoglobin (35). The error in the nitrogen determination was less than 1% of the total nitrogen present.

Electrophoretic analysis was conducted in a constant current Tiselius apparatus as modified by Longsworth (23). Mobilities of the peaks were calculated by standard methods (1) from the migration distances of the descending boundary read directly from the plates. All peaks could usually be recognized on both ascending and descending boundaries, although, as usual, resolution was better on the ascending side.

RESULTS

Molecular Weight of Unoxidized and Oxidized Globin by Light Scattering

Preliminary investigation showed that above pH 2.5, aggregation of unoxidized and oxidized globin was considerable. Even at pH 2.5, in 0.2 M NaCl the negative second virial coefficient of the plot of Kc/R_{90} against concentration for unoxidized globin showed that interparticle attractive forces are greater than the Coulomb repulsion (Fig. 1). The apparent molecular weight under these conditions was 39,000. In 0.05 M NaCl at pH 2.65 for the same material the coefficient is slightly positive and the apparent molecular weight was 37,000 (Fig. 1). Fig. 2 shows corresponding data for unoxidized globin at pH 1.5 and 2.0 in 0.05 M sodium chloride. Taking the widest reasonable range of estimates of the intercepts, the molecular weight remains at about 21,000–23,000. Net interactions are not large as shown by the small second virial coefficient. Light scattering data for the oxidized globin in 0.05 M NaCl at pH 2.5 and 2.0 are shown in Fig. 3. At pH 2.5 the weight average molecular weight of the oxidized globin, 41,000, is approximately equal to that of the unoxidized material. However, contrary to the results for unoxidized globin, no further decrease in molecular weight was apparent at pH 2.0.

Molecular Weight of Hemoglobin, and of Unoxidized and Oxidized Globin by Osmotic Pressure

Osmotic pressure data for hemoglobin and oxidized globin at pH 1.8, and for unoxidized globin at pH 2.0, in 0.05 M NaCl are given in Fig. 4. The number average molecular weight of hemoglobin from the extrapolated value of P/cRT was $20{,}000 \pm 1000$, of oxidized globin about 21,000, and of unoxidized globin $16{,}000 \pm 1000$.

Velocity Sedimentation

Above pH 2.5 or in salt solutions above 0.05 M the sedimenting boundaries of solutions of unoxidized and oxidized globin became skew or separated into

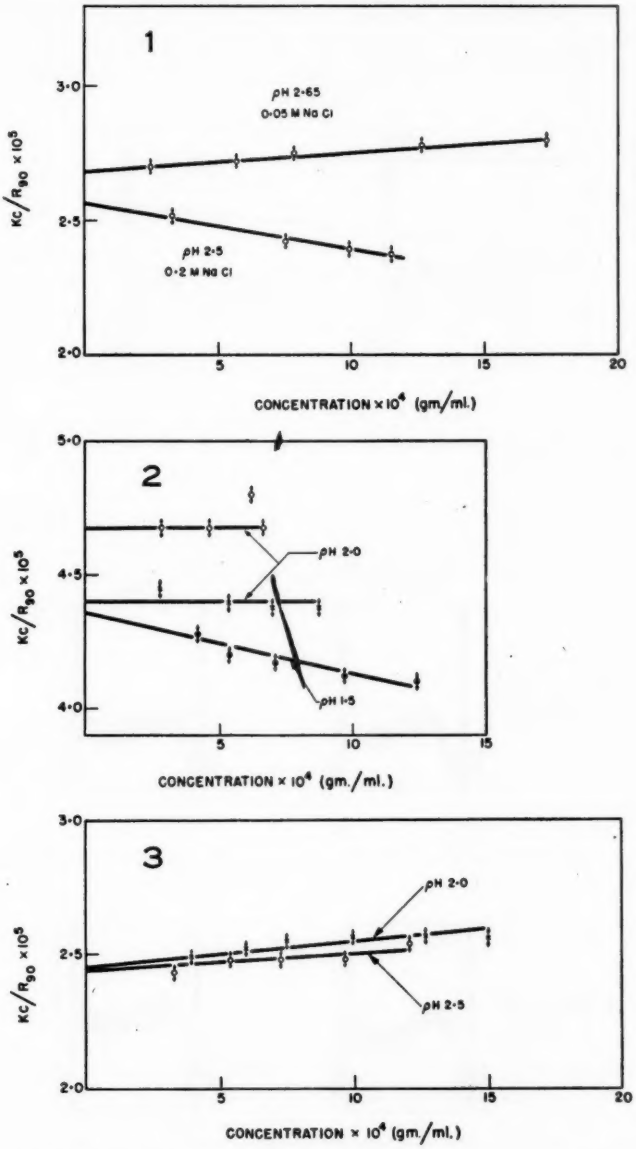

FIG. 1. Light scattering data for unoxidized globin, under two conditions of sodium chloride and hydrogen ion concentration.

FIG. 2. Light scattering data for unoxidized globin in 0.05 *M* sodium chloride at pH 1.5 and 2.0. The points shown for experiments at pH 2.0 give the maximum range observed.

FIG. 3. Light scattering data for oxidized globin in 0.05 *M* sodium chloride.

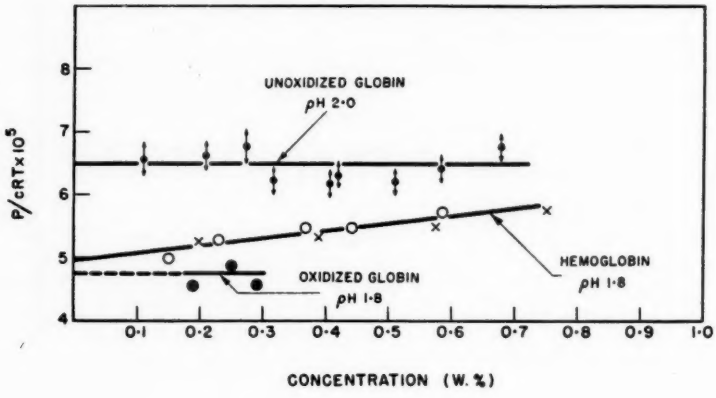

FIG. 4. Osmotic pressure data for hemoglobin, and for unoxidized and oxidized globin in 0.05 *M* sodium chloride.

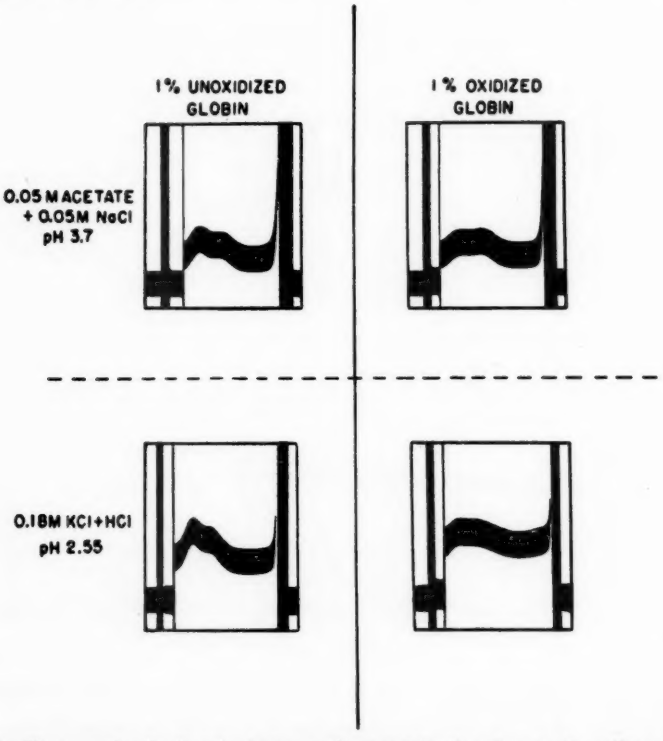

FIG. 5. Photographs of refractive index gradient distributions in velocity sedimentation of unoxidized and oxidized globin under two conditions.

poorly resolved peaks (Fig. 5). These features disappeared progressively upon decreasing the pH or salt concentration, thereby eliminating the possibility that they were evidence for stable components. At or below about pH

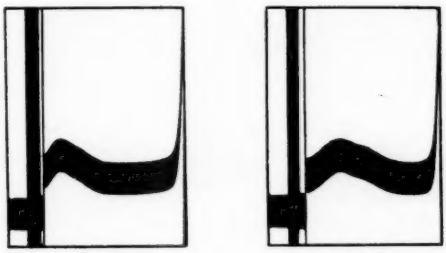

FIG. 6. Photographs of refractive index gradient distributions of unoxidized globin in 0.05 *M* sodium chloride.

2.5, the sedimenting peak for unoxidized globin was single, broad, and less asymmetric (Fig. 6) and the sedimentation coefficient was constant within experimental error (Table I). Its value is approximately one half that given by Gralén for the same material at pH 4.0 (13).

TABLE I

THE SEDIMENTATION COEFFICIENT OF UNOXIDIZED HORSE GLOBIN IN 0.05 *M* NaCl AS A FUNCTION OF pH

pH	Concentration, %	$S_{w,20}$
1.50	0.8	1.08×10^{-13}
1.65	1.0	1.20
2.05	1.0	1.09
2.10	1.0	1.18
2.50	1.0	1.29
		Mean 1.17×10^{-13}

TABLE II

THE SEDIMENTATION COEFFICIENT OF UNOXIDIZED HORSE GLOBIN IN 0.05 *M* NaCl, pH 2.5, AS A FUNCTION OF CONCENTRATION

Concentration, %	$S_{w,20}$
0.3	1.43×10^{-13}
0.5	1.30
0.7	1.38
1.0	1.29

Table II gives the sedimentation coefficient of unoxidized globin in 0.05 *M* NaCl, pH 2.5, as a function of concentration. Clearly the coefficient is not a marked function of concentration under these conditions.

Tables III and IV give the results of a determination of the sedimentation coefficient of oxidized globin as a function of pH and concentration respectively. Table IV also gives the same data for a sample of overoxidized globin.

TABLE III

THE SEDIMENTATION COEFFICIENT OF OXIDIZED GLOBIN (1.0%), IN 0.05 *M* NaCl, AS A FUNCTION OF pH

pH	$S_{w,20}$
1.65	1.65×10^{-13}
2.05	1.44
1.65	1.68
2.9	1.62

TABLE IV

THE SEDIMENTATION COEFFICIENT OF OXIDIZED AND OVEROXIDIZED GLOBIN IN 0.05 *M* NaCl, pH 2.5, AS A FUNCTION OF CONCENTRATION

Conc., %	$S_{w,20}$
Oxidized globin	
0.3	1.83×10^{-13}
0.5	1.61
0.7	1.73
	Mean 1.72×10^{-13}
Overoxidized globin	
0.3	2.87×10^{-13}
0.5	2.82
0.7	2.72
	Mean 2.80×10^{-13}

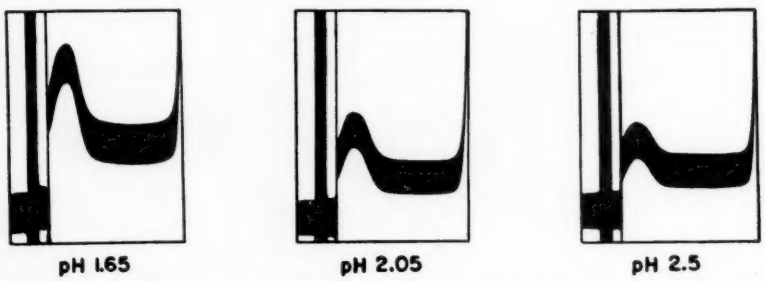

FIG. 7. Photographs of refractive index gradient distribution of oxidized globin in 0.05 *M* sodium chloride.

Typical patterns are shown in Fig. 7. The sedimentation coefficient of oxidized globin is not a strong function of either pH or concentration but the coefficient of the overoxidized sample is nearly double that of the less drastically treated one.

To assess the effect of removal of the heme groups on the sedimentation coefficient, the $S_{w,20}$ of hemoglobin was determined as a function of concentration at pH 2.5 in 0.05 *M* NaCl and in 0.2 *M* NaCl. Results are given in Table V.

TABLE V

THE SEDIMENTATION COEFFICIENT OF HORSE HEMOGLOBIN AT pH 2.5 IN 0.05 *M* NaCl AND 0.2 *M* NaCl AS A FUNCTION OF CONCENTRATION

0.05 *M*		0.20 *M*	
Conc., %	$S_{w,20}$	Conc., %	$S_{w,20}$
0.1	1.64×10^{-13}	0.1	3.26×10^{-13}
0.2	1.65	0.2	3.35
0.3	1.95	0.3	2.84

At pH 2.5 in 0.05 *M* NaCl the coefficient for hemoglobin is higher than for unoxidized globin but is comparable to that for oxidized globin (Table IV). Increasing the sodium chloride concentration to 0.2 *M* doubled the coefficient for hemoglobin.

This increase in sedimentation coefficient with increasing salt concentration may be attributed to either reassociation of subunits *or* a decrease in the electrostatic repulsion of subunits, without association. To distinguish between these possibilities, the sedimentation coefficient of ribonuclease was determined as a function of concentration in 0.05 *M* NaCl, pH 2.5. Ribonuclease was chosen because it has an isoelectric point close to that of hemoglobin (32), a molecular weight comparable with that of the suspected subunit of hemoglobin (10, 11), and is known to consist of one polypeptide chain (3). Results are given in Table VI. The mean value 1.85×10^{-13} at pH 2.5 is

TABLE VI

THE SEDIMENTATION COEFFICIENT OF RIBONUCLEASE IN 0.05 *M* NaCl, pH 2.5, AS A FUNCTION OF CONCENTRATION

Conc., %	$S_{w,20}$
0.3	1.89×10^{-13}
0.5	1.86
0.7	1.81
	Mean 1.85×10^{-13}

in excellent agreement with that for ribonuclease close to neutrality, 1.85×10^{-13} (11). These results suggest that the direct electrostatic effects upon the sedi-

mentation coefficient of hemoglobin and globin may be neglected, even under these rather drastic conditions and that the increasing sedimentation coefficient reflects increasing aggregation.

Equilibrium Sedimentation

The results of applying Archibald's procedure (5), for estimating equilibrium ultracentrifuge molecular weights, to unoxidized globin in 0.05 *M* NaCl, pH 2.0, are summarized in Fig. 8. The estimated values of δ are equal at the base and at the meniscus of the cell and correspond to a molecular weight of 22,000. Hemoglobin under the same conditions was polydisperse with an apparent molecular weight of about 23,000 at the meniscus and 50,000 at the base.

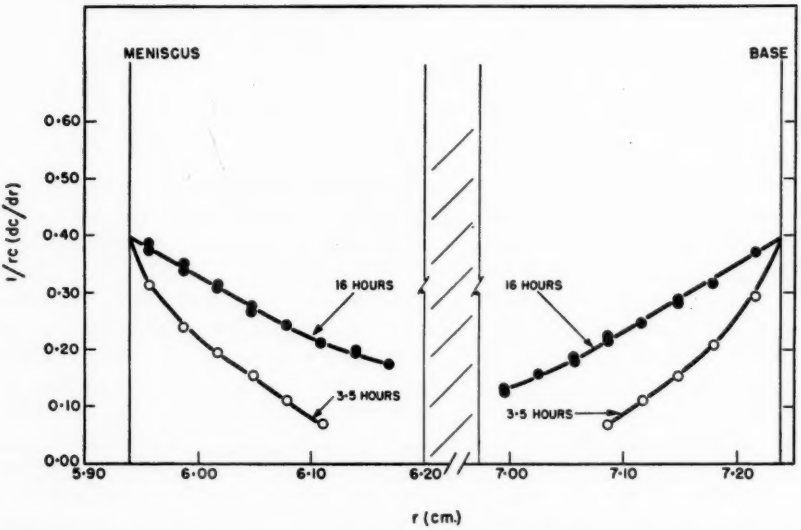

FIG. 8. Archibald's approach to equilibrium sedimentation for unoxidized globin in 0.05 *M* sodium chloride, pH 2.0.

Electrophoresis

Unoxidized and oxidized globin showed similar complex behavior when examined electrophoretically from pH 4.0 to pH 1.5 in dilute buffer or salt solutions. Resolution of the apparent components was better in the unoxidized material, however, presumably because of a decrease in the isoelectric point of the oxidized globin imposed by newly introduced negative groups. Increasing the salt concentration from 0.05 *M* to 0.2 *M* NaCl decreased resolution of some peaks in the pattern or eliminated them completely. At pH 4.0 in 0.05 acetate and 0.05 *M* NaCl, one large peak was observed, accompanied by a slower, smaller component (Fig. 9). In some experiments, this slower peak could not be resolved and only one peak was apparent, particularly at higher salt con-

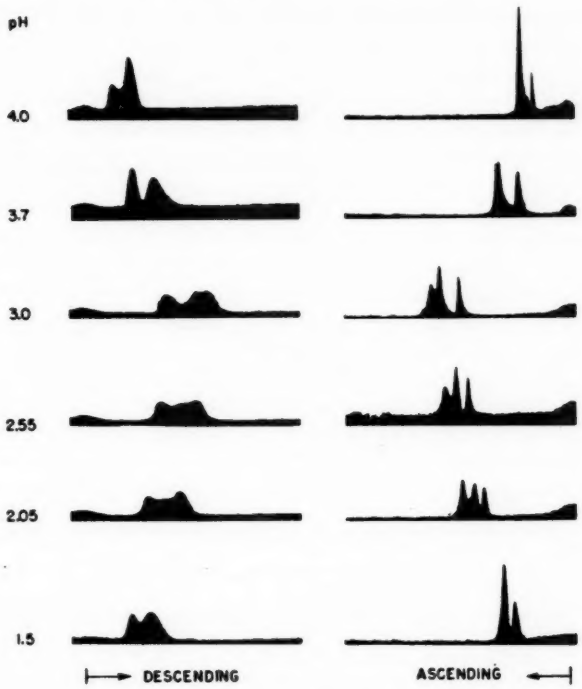

FIG. 9. Electrophoretic patterns of unoxidized globin in 0.05 *M* sodium chloride as a function of pH. At pH 3.7 and 4.0, the solutions contained 0.05 *M* acetate in addition to the sodium chloride.

centrations. As the pH decreased to 3.7 the apparent proportion of this slower component increased until the concentration of both components was approximately equal. These were presumed to be the two components previously observed in the hemoglobin of other species (18, 25, 30) at high hydrogen ion concentrations. A further decrease in pH was attended by separation of a third, faster component until at pH 2.5 the apparent concentrations of all three were approximately equal. Finally between pH 2.0 and 1.5, one component disappeared leaving only two well-defined peaks. Similar variation of components with pH was shown by the untreated horse hemoglobin. Approximate estimates of the mobilities of the three components of unoxidized globin in the descending boundary are given in Fig. 10 as a function of pH. Within experimental error the mobility of the intermediate peak remains constant from pH 1.5 to 4.0 while that of the slowest goes through a maximum at about pH 3.0. The mobility of the fastest peak apparently decreases from pH 3.0 where it is first evident to pH 2.0 below which it either disappears or incorporates all of the material of intermediate mobility.

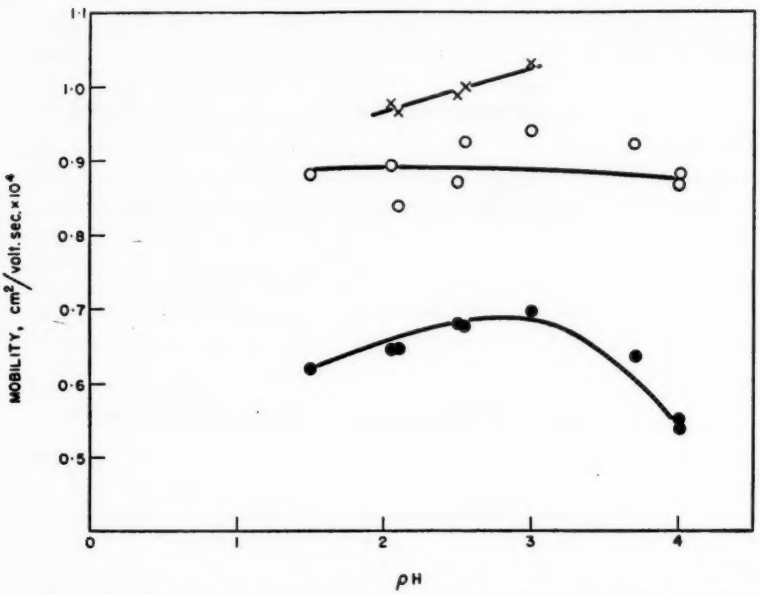

FIG. 10. Estimates of the mobilities of the three apparent components of unoxidized globin as a function of pH.

DISCUSSION

The foregoing data from light scattering, osmotic pressure, and sedimentation velocity show that a net effect of oxidation of globin by performic acid is to *increase* the molecular weight of the material, at a given pH level, rather than decrease it, as would be expected if covalent bonds were being broken. The increase therefore implies that disulphide bonds do not link polypeptide chains in horse hemoglobin, in agreement with conclusions from sulphydryl determinations (21). The higher molecular weight may be attributed to reassociation of the subunits of hemoglobin, following reduction of their isoelectric points by the additional sulphonic acid groups. Since horse hemoglobin contains a negligible percentage of phosphorus (18, 38) it is therefore probable that the polypeptide chains are held together in the molecule only by secondary bonds or by mechanical entanglement.

The difference between the number average molecular weight and the weight average value for unoxidized globin in 0.05 *M* NaCl at pH 1.8–2.0, together with the ill-defined peaks and rapid spreading of the sedimenting boundary in the ultracentrifuge at higher pH levels, shows that the solutions are polydisperse. This heterogeneity must reflect an association of subunits of approximately equal size rather than a collection of independent particles of different molecular weights because (*a*) sedimentation velocity boundaries increase in symmetry as the pH decreases, (*b*) only the assumption of dissociation products of equal size is compatible simultaneously with the observed

molecular weights and the observation that the products do not pass a membrane which is readily permeable to ribonuclease. At pH 2.0 in 0.05 *M* NaCl however, the equilibrium point is far toward the side of dissociation as shown by the lack of effect of concentration change on $S_{w,20}$. For this reason, the observed number average molecular weight at pH 2.0, 16,000–17,000, will be a good approximation to the molecular weight of the dissociation products and indicates that the total number of dissociable subunits in the hemoglobin molecule under these conditions is four. A smaller number cannot account for the observed osmotic pressures and a larger number is incompatible with the retention of all the dissociation products by the membrane. The conclusion of four subunits of equal size in hemoglobin is likewise consistent with the approximately fourfold reduction in sedimentation coefficient of hemoglobin at pH 7.0 to that of globin at pH 2.0. These results therefore directly confirm previous inferences.* No evidence for further dissociation was found, even at pH 1.5. Consequently, unless two polypeptide chains are so closely intertwined as to prevent separation under these drastic conditions, Porter and Sanger's observation of six N-terminal amino acid residues in horse hemoglobin suggests that branching occurs in at least two of the polypeptide chains which comprise the subunits.

The equilibrium in the association between these subunits is not established instantaneously, as shown by the presence of transient and poorly resolved peaks in velocity sedimentation. It is complete, however, in 3.5 hr. at pH 2.0 as shown by the equality of δ for unoxidized globin at the base and the meniscus of the cell in the approach to equilibrium ultracentrifugation. Field and O'Brien have recently shown that the dissociation to half molecules is rapid in acid solution for human hemoglobin (12), in which the extent of splitting is much less than for horse hemoglobin (37). Consequently, these observations suggest that the dissociation of the horse hemoglobin molecule in acid solution takes place in two distinct stages under the influence of Coulomb repulsion; first a rapid, reversible dissociation to half molecules followed by a slower, not completely reversible separation of the components of the half molecules. This latter is probably the process to which the term denaturation has been applied (12).

As shown by velocity sedimentation, equilibrium sedimentation, and osmotic pressure experiments, heme tends to stabilize the association of the subunits of globin, even at pH levels far below 3.9, at which point the porphyrin ring begins to split from the protein (20).

The electrophoretic analyses show that the two half molecules of horse hemoglobin differ electrokinetically and also provide additional qualitative evidence for further splitting of half molecules into distinct subunits. No exact definitive interpretation of the patterns can be given but they are consistent with the assumption that at pH 4.0–3.7, the hemoglobin splits predominantly into half molecules of different mobilities. As the pH decreases,

**Gutter and Peterson have also very recently found indirect evidence for four subunits in horse hemoglobin from studies on the effect of mercaptoethanol and urea on the sedimentation rate, as reported at the American Chemical Society Meeting, Sept. 13, 1955, Minneapolis, Minn.*

an increasing proportion of both these half molecules dissociate to subunits. Of these four subunits, at least two must differ electrokinetically. If a larger number differ, they may reassociate to form new complexes of intermediate mobility. Such complexes would be an adequate explanation for the discordant observations reported on similar preparations of hemoglobins from other species (25, 30). Finally, at very low pH's (i.e. below 2.0), Coulomb repulsion is so strong between subunits that complex formation is repressed and the apparent number of components decreases.

One over-all conclusion from these experiments must be stressed. The tendency to association between the different subunits of the hemoglobin molecule is surprisingly strong, in spite of the absence of any covalent bonds and in opposition to Coulomb repulsion. This must confer a stability to the general configuration of the hemoglobin molecule, which may play a role in its physiological function.

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SOME XANTHATE METHYL ESTERS OF GLUCOSE[1]

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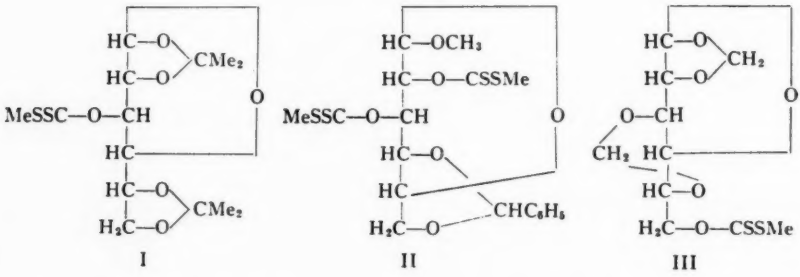
ABSTRACT

The following compounds were thought to be new: 1,2-mono-*O*-isopropylidene-D-glucofuranose-3-*S*-methyl xanthate, m.p. 102°, $[\alpha]_D^{24}$ −27.8°; methyl-4,6-*O*-benzylidene-α-D-glucopyranoside-2,3-di-*S*-methyl xanthate, m.p. 100°, $[\alpha]_D^{22}$ −18.1°; and 1,2;3,5-di-*O*-methylene-α-D-glucofuranose-6-*S*-methyl xanthate, m.p. 99°, $[\alpha]_D^{21}$ +27.3° in chloroform. Partial hydrolysis of the isopropylidene and methylene derivatives yielded some glucose-3-*S*-methyl xanthate and glucose-6-*S*-methyl xanthate as crude sirups. The 6-xanthate greatly excelled the 3-xanthate in stability toward acid. The chromatographic behavior of both was determined.

INTRODUCTION

Although xanthate salts are very readily decomposed by acid, a recent research (20) confirmed the claim that the *S*-methyl xanthate esters of simple alcohols are often remarkably stable in acidic conditions. The location of the substituents in cellulose sodium xanthates (viscose) might therefore be determined by conversion to the *S*-methyl esters, hydrolysis to the corresponding glucose derivatives, and identification of the latter. In order to explore this possibility, attempts have now been made to prepare some of the relevant glucose-*S*-methyl xanthates, to determine their stability toward acids and their behavior in chromatography.

In 1927 Freudenberg and Wolf (4) shook 3-sodio-1,2;4,5-di-*O*-isopropylidene glucofuranose first with carbon disulphide to form the 3-xanthate salt, and then with methyl iodide to obtain the crystalline 3-*S*-methyl ester (I). They



I II III

apparently did not succeed in selectively hydrolyzing the isopropylidene groups and therefore recovered neither glucose-3-*S*-methyl xanthate nor its monoisopropylidene derivative. A repetition of this work showed that the yield of I could be increased from 52% to nearly 90% of theory, based on the

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diisopropylidene glucose that reacted. The substance (I) was then submitted to acetolysis in an attempt to replace the isopropylidene by acetyl groups, as Hann, Hudson, and their numerous collaborators succeeded in doing with similar cyclic acetals (6, 13, 22). Although analyses suggested that some of the isopropylidene had indeed been replaced by acetyl groups, the impure product failed to crystallize, and eventually the method was abandoned. The diisopropylidene methyl xanthate was then hydrolyzed with concentrated nitric acid in ethyl acetate as described by Coles, Goodhue, and Hixon (2) for the removal of the 5,6-isopropylidene group from 1,2;5,6-di-*O*-isopropylidene glucofuranose, but some xanthate groups were lost and the product was an impure sirup. The realization that this xanthate ester was not nearly so resistant toward acidic reagents as those studied by Vincent (20) led to an attempt to prepare a more stable derivative by oxidizing I with 30% aqueous hydrogen peroxide in glacial acetic acid. Unlike Vincent's experience with octadecyl-*S*-methyl xanthate, this oxidation failed to yield a stable crystalline derivative retaining all of the original sulphur, but produced extensive decomposition. Details of these and other negative experiments have been omitted from this account.

Freudenberg, Dürr, and Hochstetter (3) found that boiling methanolic hydrogen chloride removed the isopropylidene group in the 5,6-position of diisopropylidene glucose 40 times more rapidly than the one in the 1,2-position. By restricting the action of a 0.15% hydrogen chloride solution to eight minutes, it was found possible to prepare a pure, crystalline mono-*O*-isopropylidene glucose-3-*S*-methyl xanthate from the diisopropylidene derivative in about 35% yield, 30% of the starting material being recovered unchanged. When chromatographed on paper, using *n*-butanol saturated with water as the solvent and ammoniacal silver nitrate as the spray, the mono- and diisopropylidene methyl xanthates had R_f values of 0.92 and 0.97, respectively. The former compound produced a yellow spot with the spray immediately, but the latter developed a dark gray spot only after the paper had been heated at 100° for a few minutes. The two compounds could therefore be distinguished readily.

In order to study the hydrolysis of the monoisopropylidene methyl xanthate, a 2% solution in 0.05 *N* aqueous hydrochloric acid was boiled under reflux and the change in optical rotation was observed at intervals. After 105 min. the xanthate group was obviously decomposing but the specific rotation of 22.5° was not far from the value of 13° roughly estimated for glucose-3-*S*-methyl xanthate from the molecular rotations of its monoisopropylidene derivative, of 1,2-*O*-isopropylidene glucose, and of glucose, in water or alcohol. The crude product when chromatographed yielded faint spots corresponding to glucose (R_f 0.20), to the isopropylidene methyl xanthate (R_f 0.92), and to an unknown substance. The major spot, with R_f 0.65, was attributed to the desired glucose-3-*S*-methyl xanthate. This compound partly decomposed when an attempt was made to free it from the associated glucose by a fermentation with yeast (16). A product whose purity was estimated from its sulphur content to be about 90% resulted when monoisopropylidene glucose methyl xanthate was boiled

for two hours in 50% methanol containing 1% of sulphuric acid, which was then neutralized with barium carbonate. Some of the starting material, 54%, was recovered unchanged, and the crude glucose-3-*S*-methyl xanthate was isolated by evaporating the frozen aqueous liquor. The most satisfactory method of preparing glucose-3-*S*-methyl xanthate proved to be the partial hydrolysis of the more accessible diisopropylidene compound (I) in hot 80% acetic acid, which was used by Ness, Hann, and Hudson (12) in a similar case. Although 55% of the monoisopropylidene derivative was recovered, it was easy to eliminate the acetic acid and to purify the glucose-3-*S*-methyl xanthate by chromatography on a cellulose column. The product gave only a single spot of R_f 0.65 when chromatographed on paper, but nevertheless persisted as an uncrystallized, light yellow oil containing somewhat less than the calculated amount of sulphur.

The xanthation of methyl-4,6-*O*-benzylidene-α-D-glucoside was next undertaken. Although attempts to prepare the intermediate 2,3-di-sodio derivative with sodium in liquid ammonia according to Muskat (11) were not successful, the derivative apparently formed in high yield when a solution of the benzylidene compound in dioxane was mixed with a colloidal suspension of sodium in toluene. The subsequent addition of carbon disulphide to the suspension, followed still later by the addition of methyl iodide, produced crude, crystalline methyl 4,6-*O*-benzylidene-α-D-glucopyranoside-2,3-di-*S*-methyl xanthate (II) in about 70% yield. All attempts to remove the benzylidene group, or the benzylidene and methyl glucosidic groups, selectively led to yellow, evil-smelling oils containing less sulphur than the amounts expected for glucose-2,3-di-*S*-methyl xanthate or for the corresponding methyl glucoside. No single substance could be detected in these oils by the chromatographic methods used, and the attempt to prepare glucose-2,3-di-*S*-methyl xanthate was abandoned owing to the instability of the xanthate ester groups.

The most suitable compound from which to attempt a synthesis of glucose-6-*S*-methyl xanthate appeared to be the di-*O*-methylene glucose-6-acetate synthesized by Hough, Jones, and Magson (7), which was characterized as the 1,2;3,5-di-*O*-acetal by Schmidt, Distelmaier, and Reinhard (17) and later by Shyluk, Honeyman, and Timell (18). The replacement of the *O*-acetyl group by a sodium atom proceeded smoothly in alcoholic sodium hydroxide, and subsequent treatments with carbon disulphide and with methyl iodide produced the new, crystalline 1,2;3,5-di-*O*-methylene glucofuranose-6-*S*-methyl xanthate (III) in 84% yield. This compound was difficult to hydrolyze and the methylene and xanthate ester groups appeared to be removed at roughly the same rate. The most economic procedure found for the preparation of glucose-6-*S*-methyl xanthate was to boil a solution of the dimethylene derivative in 6% aqueous-alcoholic sulphuric acid for about three hours; about 80% of the starting material was recovered and recycled, and the hydrolyzates were accumulated. When these hydrolyzates were chromatographed on a cellulose column, a substance was isolated which had a single R_f value of 0.84 in the system used, and a sulphur content close to that required by glucose-6-*S*-methyl xanthate (S, 23.7%). All attempts to crystallize the

product, or its tetraacetate, failed. In addition to glucose (R_f 0.13) the hydrolyzate contained a minor amount of a sirup containing 9.2% of sulphur and with an R_f value of 0.70. This sirup was presumably a decomposition product of the methyl xanthate, because the product from a similar hydrolysis prolonged for 68 hr., when chromatographed on paper, yielded the spot expected for glucose, and others with R_f values of 0.30, 0.56, and 0.70, the one at R_f 0.84 being absent.

Ness, Hann, and Hudson (13) showed that acetolysis replaced the methylene acetal rings in a trimethylene mannitol with acetyl and acetoxymethyl groups, while Lieser and Leckzyck (10) found that acetolysis at 0° did not affect a xanthate group. By combining the techniques used in these researches, it proved possible to cleave di-*O*-methylene-glucose-6-acetate to a presumed tri-*O*-acetyl-di-*O*-acetoxymethyl derivative and to deacetylate the latter to glucose. A repetition of the work with the xanthate ester (III) produced a sirup with the composition of a diacetyl diacetoxymethyl glucose methyl xanthate, but attempts to deacylate this substance, even with very mild conditions, resulted in some dexanthation. According to a paper chromatogram, the product contained a little glucose, much of the 6-*S*-methyl xanthate of R_f 0.84, and a little of the related substance of R_f 0.70.

The *S*-methyl xanthates (I), (II), and (III) were also hydrolyzed with cold concentrated hydrochloric acid in conditions suitable for the complete hydrolysis of a cellulose-*S*-methyl xanthate to the corresponding glucose derivatives. As expected, the 3-*S*-methyl xanthate group in (I) was completely removed and nothing but glucose was detected in the product; no substance was recognized in the hydrolyzate of the 2,3-di-*S*-methyl xanthate (II), but the 6-*S*-methyl xanthate (III) yielded 70% of a sirup containing glucose, glucose-6-*S*-methyl xanthate (R_f 0.84), and the derived substance of R_f 0.70. These experiments confirmed the observation that an *S*-methyl xanthate group located in the sixth position of glucose was much more stable toward acid than were those in the second and third positions.

EXPERIMENTAL

Analytical

Sulphur was determined according to Waters (21) by oxidizing 0.05 to 0.1 gm. samples with bromine – nitric acid and estimating the resulting sulphuric acid as the barium salt. Microdeterminations on 1–5 mgm. samples containing 10–20% sulphur were fairly accurate when the Grote combustion to sulphur dioxide was used, as described by Sundberg and Royer (19). The dioxide was oxidized by 3% aqueous hydrogen peroxide to sulphuric acid, which was increased in amount by the addition of exactly 10 ml. of 0.01 *N* acid before being titrated to exactly pH 5.5 with 0.004 *N* sodium hydroxide. The determination of acetyl groups by saponification (9) was inaccurate in presence of *S*-methyl xanthates, but Clark's saponification followed by steam-distillation (1) gave good results. The almost complete failure of the *S*-methyl xanthate group to yield methyl iodide (20) in a customary determination of alkoxyl groups (15) was confirmed.

Techniques used in paper chromatography were based on the description given by Partridge (14), butanol saturated with water being the solvent and ammoniacal silver nitrate usually being the spray.

*1,2;5,6-Di-O-isopropylidene-*D*-glucofuranose-3-S-methyl Xanthate (I)*

The 1,2;5,6-di-*O*-isopropylidene glucofuranose was prepared from glucose, acetone, anhydrous zinc chloride, and phosphoric acid as described by Glen, Myers, and Grant (5). After several recrystallizations, the melting point and specific rotation in water attained the correct values of 112–113° and $[\alpha]_D^{20}$ −18.8° (*c*, 2.04), respectively.

An excess of sodium in the form of thin plates was added in nine installments during 24 hr. to a solution of 50 gm. of diisopropylidene glucose in 200 ml. of dry ether kept under reflux on a steam bath. The ether solution was decanted and the residual sodium was washed three times with ether. The solution and washings containing the sodio-derivative were combined, cooled, mixed with 50 ml. of carbon disulphide, and kept at room temperature for 12 hr. The ether layer containing any unchanged diacetone glucose was then decanted, and the residual semisolid, cream-colored mass of crude xanthate salt was mixed with 15 ml. of pure methyl iodide. After being vigorously shaken at intervals during an hour to disintegrate the mass, the suspension was kept near 20° for six hours and was then filtered. The combined filtrate and washings were evaporated under nitrogen, and the solid residue was distilled at 150–160° and 0.6–0.7 mm. pressure. When a solution of the yellow distillate in ether was allowed to evaporate slowly, 61 gm. of crystals melting at 57–60° separated. This yield of the *S*-methyl xanthate amounted to 90% of theory, instead of 52% as reported by Freudenberg and Wolf (4). After recrystallization from petroleum ether the substance had the correct sulphur content, melted at 58–60°, and had a specific levo rotation of $[\alpha]_D^{22}$ −15.5° in chloroform (*c*, 2.00). The recorded values (4) were m.p. 61° and $[\alpha]_{6460}^{16}$ −14.6° in acetylene tetrachloride.

One gram of the crystalline methyl xanthate dissolved within five minutes when shaken at room temperature with 50 ml. of 37% hydrochloric acid. After six hours, when the solution had become dark red and developed an unpleasant odor, an equal volume of water was added. Six days later, and after further dilution with water, the acid was neutralized with basic lead carbonate and evaporated to dryness under diminished pressure. Extraction of the residue with five 25 ml. volumes of ethanol removed 0.5 gm. of a red sirup, which when chromatographed on paper gave a single spot corresponding to glucose.

*Mono-O-isopropylidene-*D*-glucofuranose-3-S-methyl Xanthate*

(*a*) A solution of 135 gm. of the diisopropylidene derivative in 140 ml. of absolute methanol containing 0.15% of anhydrous hydrogen chloride was heated under reflux for eight minutes. The solution was cooled, neutralized with basic lead carbonate, and filtered, lead being later removed as the sulphide from the filtrate. After being evaporated near 20° and under reduced pressure, the clear liquor left a residue which partly crystallized within two days. This

residue was dissolved in hot ligroin (b.p. 80–110°) and the solution on cooling deposited 46 gm. (38.5%) of the product, m.p. 94–98°. Approximately 45 gm. (30%) of the unchanged diisopropylidene methyl xanthate was recovered from the mother liquor. A 3.3 gm. sample of the mono derivative when thrice recrystallized from 200 to 300 ml. of hot ligroin yielded 2 gm. of pure material melting at 101–102.5°. The specific levorotation was $[\alpha]_D^{24}$ −27.8° in chloroform (*c*, 2.00). Found: C, 42.5, 42.5; H, 5.9, 6.0; S, 20.7, 21.0%. Calc. for $C_{11}H_{18}O_6S_2$: C, 42.6; H, 5.8; S, 20.6%.

When a similar methanolysis of the diisopropylidene derivative was continued for about one hour, a 60–70% yield by weight was obtained of a white, evil-smelling, amorphous material which became liquid and discolored when exposed to the air. The substance was insoluble in ether but soluble in methanol. Found: S, 20.1; OCH_3, 2.8, 2.2%. The low value for apparent methoxyl made the formation of any methyl glycoside very doubtful.

Some of the crystalline monoisopropylidene glucose-*S*-methyl xanthate, 0.5 gm., was dissolved in 25 ml. of 0.05 *N* hydrochloric acid in aqueous ethanol (1 vol.: 1 vol.). Since the optical rotation as observed in a 2 dm. tube did not change within 12 hr. at room temperature, the solution was heated under reflux on a steam bath. After 0, 15, 30, 60, 75, and 105 min. the observed rotations corresponded to specific rotations of −27.5°, −12.5°, −3.8°, +5.0°, +7.5°, and +22.5°. The xanthate ester group was then decomposing, to judge from the odor and turbidity of the solution. The removal of hydrochloric acid from this solution by silver carbonate caused immediate blackening even at room temperature, and the use of Amberlite IR-4B anion exchange resin also removed half of the sulphur. The odor of mercaptan was strong when this resin was regenerated, first with alkali and then with acid. Basic lead carbonate was finally adopted as the neutralizing agent because no blackening occurred unless the solution was warmed. The lead was later removed as the sulphide, the clear filtrate was extracted with chloroform to recover most of the residual monoisopropylidene-glucose-*S*-methyl xanthate, and the aqueous phase was chromatographed on paper with the results already discussed.

(*b*) Following the method described by Ness, Hann, and Hudson (12), 2 gm. of diisopropylidene glucose-3-*S*-methyl xanthate was dissolved in 50 ml. of 80% acetic acid. The solution was heated under reflux for 30 min. in an oil bath kept at 120°, and was then evaporated to a sirup under reduced pressure. This sirup was extracted with 30 ml. of chloroform, and the dried extract on evaporation yielded 1.1 gm. (55%) of material which solidified on being stirred with ligroin. The solid, m.p. 75–88°, was shown by chromatographic methods to be the monoisopropylidene methyl xanthate contaminated with a small amount of the diisopropylidene derivative. The portion, 0.4 gm., of the above sirup which was insoluble in chloroform was saved (see below).

Glucose-3-S-methyl Xanthate

A solution of the above 0.4 gm. of sirup in 25 ml. of acetone was filtered, and a portion of the clear filtrate was chromatographed on paper. A small amount of glucose appeared to be present, together with a large amount of

the compound supposed to be glucose-3-*S*-methyl xanthate and a trace of a third substance of intermediate R_f value. An accumulation of 4.5 gm. of the sirup from several hydrolyses, and which had an average sulphur content of 20.45%, was then purified by passage through a cellulose column. The column was eluted with 1200 ml. of *n*-butanol saturated with water, and the eluate was collected in 21 fractions. Fractions Nos. 11 to 17 inclusive gave only one spot with an R_f value of 0.65 on a paper chromatogram. These fractions when combined and evaporated yielded 3 gm. of glucose-3-*S*-methyl xanthate as a light yellow sirup. Found: S, 20.1, 20.2%. Calc. for $C_8H_{14}O_6S_2$: S, 23.7%. The compound was not quite pure, and repeated attempts to crystallize it failed.

Methyl-4,6-O-benzylidene-α-D-glucopyranoside-2,3-di-S-methyl Xanthate

α-Methyl-D-glucoside and benzaldehyde were heated together as described by Irvine and Scott (8), and the resulting benzylidene derivative melted correctly at 163–164°. Eight grams of sodium, colloidally dispersed in 50% concentration in toluene, was added to a solution containing 8 gm. of methyl-4,6-benzylidene-α-D-glucoside in 50 ml. of dry, pure dioxane. The replacement of the dioxane by 800 ml. of dry benzene, or of the sodium by alcoholic sodium methylate followed by evaporation, was not successful. After being cooled in order to moderate the initial evolution of hydrogen, the suspension was kept warm on a steam bath for three hours, when a further 2 gm. of the dispersed sodium was added and heating was continued for eight hours. The suspension was then cooled, well mixed with 25 ml. of carbon disulphide, and kept overnight at room temperature to form the sodium xanthate. Methyl iodide, 9 ml., was added and after being kept for seven hours at room temperature the suspension was filtered free of sodium salts. The latter were washed three times with dry benzene, after which the filtrate and washings were evaporated under diminished pressure, finally in a desiccator. The red, viscous residue soon crystallized, yielding 9.2 gm. (72%) of crude methyl-4,6-*O*-benzylidene-α-D-glucoside-2,3-di-*S*-methyl xanthate melting at 65–84°. Two recrystallizations from 75% ethanol, followed by an extraction with ligroin and a recrystallization from 80% ethanol, left 4 gm. of the product as pure white crystals melting at 99–100.5°. The substance had a specific rotation of $[\alpha]_D^{22}$ −18.1° in chloroform (*c*, 1.66). Found: C, 46.9, 47.3; H, 4.7, 5.0; S, 27.3, 27.4%. Calc. for $C_{18}H_{22}O_6S_4$: C, 46.8; H, 4.8; S, 27.7%.

When chromatographed on paper, the dixanthate ester formed an intense spot near the solvent front. The parent substance, methyl benzylidene glucoside, left no record on this chromatogram. A 0.5 gm. sample of the dixanthate ester was then hydrolyzed with concentrated hydrochloric acid as described for the diisopropylidene glucose 3-*S*-methyl xanthate, and 0.23 gm. of an amorphous solid was recovered. Found: S, 10.3, 9.9%. When aniline phthalate was the spray, this solid produced no spot on a paper chromatogram, and presumably contained no glucose. Another hydrolysis involved heating a solution of 1 gm. of the dixanthate ester in a mixture of ethanol, 160 ml., water, 30 ml., and concentrated sulphuric acid, 6 ml., under reflux on the steam bath for two hours. After neutralization to pH 6 and complete evapo-

ration, the liquor yielded salts and 0.3 gm. of a yellow sirup soluble in chloroform. Found: S, 16.0, 16.1%. This sirup when chromatographed on paper gave one spot of R_f 0.87, ammoniacal silver nitrate being the spray. No spot was developed with an aniline phthalate spray, as was the case when substituted glucose-3 and 6-*S*-methyl xanthates were hydrolyzed.

*1,2;3,5-Di-O-methylene-α-*D*-glucofuranose-6-S-methyl Xanthate*

The procedure of Hough, Jones, and Magson (7) was used to prepare the corresponding 6-acetate from glucose, 162 gm., paraformaldehyde, 165 gm., glacial acetic acid, 500 ml., and concentrated sulphuric acid, 75 ml. The crude, partly crystallized product, 83 gm. (37%), on recrystallization from methanol yielded 32 gm. of pure material melting correctly at 103–105°.

Five grams of sodium hydroxide, 25 gm. of the bismethylene-6-*O*-acetylglucose, and 50 ml. of absolute ethanol were shaken together until the bismethylene derivative went completely into solution owing to deacetylation. Water, 10–15 ml., was then added to dissolve the residual sodium hydroxide and two hours later the yellow solution was evaporated to dryness *in vacuo*, finally by the addition and evaporation of 25 ml. of benzene. The bismethylene glucose was extracted from this residue by dry benzene, 100 ml., boiling under reflux, and after the extract had cooled it was mixed with an excess of sodium colloidally dispersed in 25 ml. of dry benzene. After the initial reaction had subsided, 2 gm. of sodium dispersed in toluene was added, together with 25 ml. of benzene. The mixture was kept next day near 75° for four hours, cooled, and shaken with 25 ml. of carbon disulphide. The following day, when the formation of the sodium xanthate was judged to be complete, 30 ml. of methyl iodide was added to the suspension. After being shaken occasionally during a few hours, the suspension was filtered and the residual salts were washed three times with 25 ml. volumes of benzene. Evaporation of the filtrate and washings yielded 32 gm. (108%) of crude product melting at 77–88°, increased to 97–99° by recrystallization from 500 ml. of 70% ethanol followed by extraction with low-boiling ligroin. The yield of pure white needles was 25.1 gm., or 84%, and the specific rotation, $[\alpha]_D^{21}$ +27.3° in chloroform (*c*, 2.15). Found: C, 40.8, 41.1; H, 5.0, 5.1; S, 21.3, 21.7%. Calc. for $C_{10}H_{14}O_6S_2$: C, 40.8; H, 4.8; S, 21.8%.

A 1-gm. sample was hydrolyzed in concentrated hydrochloric acid as described for the diisopropylidene-3-*S*-methyl xanthate. A residue, 0.1 gm., (m.p. 81–92°) failed to dissolve within six hours and was removed. The cream-colored solution was then diluted with an equal volume of water and kept for six days, when the color had deepened only to light yellow. After being neutralized, this solution was evaporated to dryness, and an alcoholic extract of the residue yielded 0.7 gm. of a yellow sirup when evaporated in a desiccator. A portion of this sirup when chromatographed on paper, using an aniline phthalate spray, yielded a faint spot corresponding to glucose, and two major spots (R_f 0.84 and 0.70, see below) corresponding to glucose-6-*S*-methyl xanthate.

Glucose-6-S-methyl Xanthate

One gram of the pure bismethylene derivative was boiled under reflux after

solution in 150 ml. of a mixture made up from absolute ethanol, 160 ml., water, 30 ml., and concentrated sulphuric acid, 6 ml. Although some of the xanthate survived when the solution was boiled for 68 hr., more was hydrolyzed to glucose or was decomposed, and the time was restricted to 165 min. The solution was evaporated near 35° to 10 ml., and was diluted with 100 ml. of water to precipitate 0.8 gm. of unchanged bismethylene derivative (m.p. 91–96°). After neutralization with barium carbonate and filtration, the filtrate on evaporation yielded 0.15 gm. (17%) of a sirup. An accumulation of such sirups from several hydrolyses was extracted with acetone, and 2 gm. of an oil was recovered from the extract. This oil was eluted from a cellulose column with a total of 1400 ml. of *n*-butanol saturated with water. Of the 34 fractions of eluate collected, Nos. 2 to 8 inclusive yielded a single spot of R_f 0.84 when chromatographed on paper, with ammoniacal silver nitrate as the spray. When these fractions were combined and evaporated, 1.1 gm. of crude glucose-6-*S*-methyl xanthate remained as a yellow sirup which could not be crystallized. Found: S, 20.7, 21.4%. Calc. for $C_8H_{14}O_6S_2$: S, 23.7%. Fractions Nos. 22 to 26 inclusive when chromatographed on paper each yielded a single spot of R_f 0.71; these fractions together gave 70 mgm. of a sirup whose nature was not determined. Found: S, 9.1, 9.3%.

The supposed glucose-6-*S*-methyl xanthate, 0.65 gm. (R_f 0.84), was acetylated with 20 ml. of acetic anhydride containing 0.4 gm. of anhydrous zinc chloride for 24 hr. at room temperature. The solution was poured into ice and water, the mixture was neutralized and extracted with ether. Evaporation of the extract left a yellow sirup which was dried in a vacuum desiccator over phosphorus pentoxide. Yield 0.9 gm. or 85%. Found: S, 12.2, 12.5; acetyl, 38.0, 38.1%. Calc. for 1,2,3,4-tetra-*O*-acetyl glucopyranose-6-*S*-methyl xanthate: $C_{16}H_{22}O_{10}S_2$: S, 14.6; acetyl, 39.3%.

Acetolysis of Bismethylene-glucose-6-S-methyl Xanthate

Anhydrous zinc chloride, 0.1 gm., was added to a solution of 1 gm. of the pure xanthate ester in 5 ml. of acetic anhydride. The mixture was warmed on a steam bath and shaken occasionally until the zinc chloride completely dissolved. After being left at room temperature for 16 hr., the product was precipitated by pouring the solution into ice and water. A yellow sirup, presumably a diacetyl diacetoxymethyl glucose-6-*S*-methyl xanthate, was obtained. Yield 1.6 gm. or 90%. Found: C, 42.9, 42.9; H, 5.0, 5.2; S, 12.7, 12.0%. Calc. for $C_{18}H_{26}O_{12}S_2$: C, 43.4; H, 5.2; S, 12.8%.

In order to deacetylate the above product, an accumulation of 5.2 gm. was dissolved in 100 ml. of absolute methanol, and a total of 2 ml. of 1.5 *N* barium methylate in methanol was added at intervals to maintain the pH between 7.0 and 7.5 for four days (23). The pH was then adjusted to 5.5 with dilute sulphuric acid, and the filtrate from the precipitated barium sulphate was cautiously evaporated to a sirup weighing 2.2 gm. (70%). The pungent smell of formaldehyde was evident during this evaporation. A paper chromatogram of this sirup showed that it contained a little glucose, while an intense spot at R_f 0.84 and a light one at R_f 0.70 suggested the presence of much glucose-

6-S-methyl xanthate. Aniline phthalate was the spray. No recognizable product was obtained when the deacetylation was carried out by Kunz and Hudson's method (9) employing 0.1 *N* sodium hydroxide in aqueous acetone at $-10°$.

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PHOSPHORYLETHANOLAMINE[1]

By Erich Baer and Harvey C. Stancer

ABSTRACT

A synthetic procedure is described that gives in excellent yield pure phosphorylethanolamine. Its X-ray diffraction pattern and infrared spectrum are reported.

Phosphorylethanolamine (PE) occurs in biological materials both in the bound and in the free state. The isolation of PE from malignant tumors by Outhouse (17), working with E. J. King in the Banting and Best Department, supplied the first evidence for the existence of free PE in nature. Subsequently it was also found in the intestines of rats and rabbits (13), in calf embryo muscle (15), in brain (2, 3, 22), and in various tissues of cows (24). Phosphorylethanolamine has been obtained by synthesis in yields ranging from 20% to 67%: (*a*) by phosphorylation of ethanolamine phosphate (17) or ethanolamine (19) with phosphorus oxychloride, (*b*) by phosphorylation of ethanolamine with a mixture of phosphorus pentoxide and phosphoric acid (19), (*c*) by treatment of phosphorylchloroethanol with ammonia (19), and (*d*) by the interaction of orthophosphoric acid and ethylenimine (11). The widely differing melting points reported for PE (228° (19), 230° (17), 232°–233° (19), 240° (11), and 244° (18, 12)) indicate, however, that these procedures give mixtures of organic phosphates from which it is apparently difficult to obtain the pure substance. In view of the biological interest that phosphorylethanolamine possesses it seemed desirable to develop a procedure for its synthesis that would give in good yield pure phosphorylethanolamine. This has been accomplished by substituting N-carbobenzoxyethanolamine for ethanolamine, and using diphenylphosphoryl chloride instead of phosphorus oxychloride as phosphorylating agent, thus preventing the formation of any by-product. The resulting O-diphenylphosphoryl carbobenzoxyethanolamine is freed of its protective benzyl and phenyl groups by consecutive catalytic hydrogenolysis with palladium in ethanol, and platinum in acetic acid, respectively. This procedure gives a chromatographically pure, crystalline phosphorylethanolamine in an over-all yield of 83%. Its melting point (244°–245°) agrees well with that reported by Outhouse (18) and Clarke *et al.* (12) for phosphorylethanolamine.

Attempts to simplify our procedure by removing the phenyl and carbobenzoxy groups by hydrolysis in boiling barium hydroxide solution were not successful, although the same procedure when applied to diphenylphosphorylcholine chloride (4) gives in good yield the barium salt of phosphorylcholine chloride. The protective carbobenzoxy group can be removed selectively, however, by the method of Albertson and McKay (1), using gaseous hydrogen bromide. The O-diphenylphosphorylethanolamine hydrobromide is obtained in a yield of approximately 90%. Since this compound might prove useful as a

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Contribution from the Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario.

substrate in studies with phosphatases, its preparation is described in the experimental part.

In order to facilitate both the study of the structure and the quantitative estimation of natural phosphatides by infrared spectroscopy (14, 16, 21), the infrared spectra of pure individual phosphatides have been reported from this laboratory as these substances became available by synthesis (5, 6, 7). Possessing analytically pure specimens of phosphorylethanolamine and L-α-glycerylphosphorylethanolamine (8), both of which are of interest as moieties of cephalins and plasmalogens, their infrared spectra were recorded; these are shown in Fig. 1.

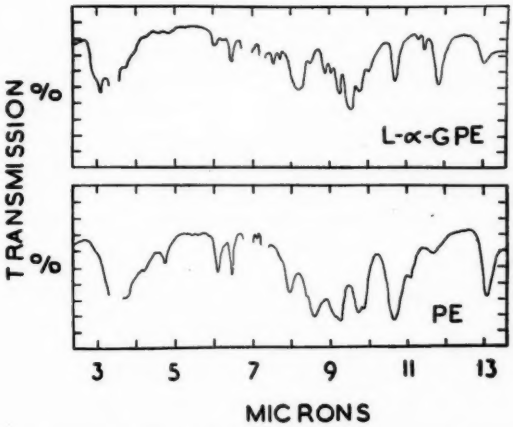
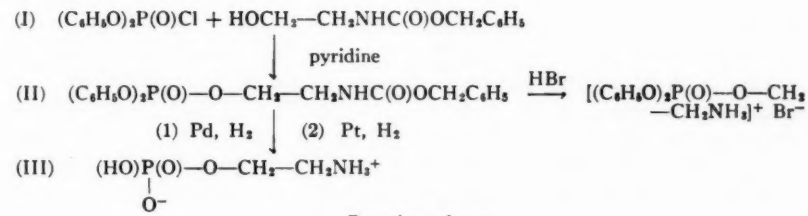


FIG. 1. Infrared spectra of phosphorylethanolamine (PE) and L-α-glycerylphosphorylethanolamine (GPE) in nujol mulls. Perkin-Elmer double-beam infrared spectrophotometer (model 21). Potassium bromide prism. Band positions and probable assignments: (PE) 2.90 μ (OH and NH stretching), 4.73, 6.08, 6.44 (NH bending), 7.10 (CH, OH bending), 7.94 (OH bending, P=O), 8.60 (C—O, C—N, C—C stretching), 9.25 (covalent phosphate), 9.72, 9.85, 10.64 (covalent phosphate), 11.08, 11.66, 13.08. (GPE) 3.10 μ (NH and OH stretching), 6.02, 6.48 (NH bending), 7.56 (CH, OH bending; primary and secondary alcohols), 7.74 (CH, OH bending; P=O), 8.18 (covalent phosphate), 8.55 (C—O, C—N, C—C stretching), 8.92, 9.08, and 9.30 (covalent phosphate), 9.58, 9.82, 10.06, 10.76 (covalent phosphate), 11.36, 11.52, 11.88, 13.06.



Reaction scheme

EXPERIMENTAL PART

O-(Diphenylphosphoryl)-carbobenzoxyethanolamine

To a solution of 9.75 gm. (5.0 mM.) of N-carbobenzoxyethanolamine (10,

20) in 40 ml. (50 mM.) of anhydrous pyridine* was added with swirling and under anhydrous conditions 14.8 gm. (5.5 mM.) of diphenylphosphoryl chloride (9) over a period of 10 min. To prevent the temperature from rising above 40°, the mixture was cooled occasionally with cold water. After it had been left for two hours at room temperature, the excess of diphenylphosphoryl chloride was destroyed by the addition of 2 ml. of water. One hour later the reaction product was isolated by precipitating it with 300 ml. of water. It was purified by washing its solution in 175 ml. of ether in succession with two 25 ml. portions of ice-cold 5 *N* sulphuric acid, and one 25 ml. portion each of water, a half-saturated solution of sodium bicarbonate, and water. After the solution had been dried over anhydrous sodium sulphate, it was concentrated under reduced pressure and the remaining solvent was removed by keeping the substance at 30°–35° in a vacuum of less than 1 mm. Hg for a period of five hours. The O-diphenylphosphoryl carbobenzoxyethanolamine, an almost colorless oil, weighed 20.4 gm. (95.4% of the theoretical amount based on carbobenzoxyethanolamine) $n_D^{23°}$ 1.5555. The substance is readily soluble at room temperature in ether, ethanol, methanol, or acetic acid but is insoluble in water. $C_{22}H_{22}O_6NP$ (427.2). Calculated: C, 61.81; H, 5.19; N, 3.29; P, 7.25. Found:† C, 61.79; H, 5.16; N, 3.30, 3.25; P, 7.12, 7.23.

Phosphorylethanolamine

A solution of 21.3 gm. (50 mM.) of O-diphenylphosphoryl carbobenzoxyethanolamine in 200 ml. of 99% ethanol together with 3.5 gm. of palladium catalyst (23) was shaken vigorously in an all-glass hydrogenation vessel of approximately 1 liter capacity in an atmosphere of pure hydrogen under a pressure of 40–50 cm. of water until the absorption of hydrogen had practically ceased. After replacing the hydrogen with nitrogen and adding 75 ml. of water to dissolve the reaction product, the catalyst was removed and the solution was brought to dryness under reduced pressure at a bath temperature of 25°–35°. To complete the removal of the protective groups, the solid residue was dissolved in 175 ml. of glacial acetic acid and the solution, after it was returned to the hydrogenation vessel and 3.5 gm. of platinic oxide‡ (Adams' catalyst) added, was shaken until there was no further uptake of hydrogen. After replacing the hydrogen with nitrogen and removing the catalyst, the acetic acid was distilled off *in vacuo* (bath temperature 30°–35°). The crude phosphorylethanolamine, containing traces of potassium, was purified by dissolving it in 350 ml. of distilled water and shaking the solution with 35 gm. of Amberlite IR–120 (H) for 90 min. The filtrate was brought to dryness *in vacuo* (bath temperature 30°–35°) and the phosphorylethanolamine was recrystallized from 35 ml. of water by gradually adding several volumes of 99% ethanol, and placing the mixture in an ice-box. The crystals were filtered off, washed with a small amount of a cold mixture of

*Pyridine of good commercial grade was refluxed over barium oxide and distilled with the exclusion of moisture.

†Analyses of two independent preparations.

‡The catalyst was prepared as described in "Organic Syntheses", Coll. Vol. 1, 2nd ed., John Wiley and Sons, Inc., New York, N. Y., 1948, p. 463, with the exception that the sodium nitrate was replaced by an equimolecular amount of potassium nitrate.

ethanol and water (2:1), and dried *in vacuo* over phosphorus pentoxide. The phosphorylethanolamine weighed 6.0 gm. (87% of theory). Over-all yield 83%, m.p. 244°–245°.* $C_2H_8O_4NP$ (141.1). Calculated: C, 17.02; H, 5.71; N, 9.93; P, 22.0. Found: C, 17.10; H, 5.60; N, 9.88; P, 22.0.

X-ray Diffraction Pattern of Phosphorylethanolamine

The X-ray powder diffraction pattern was taken using Ni-filtered Cu K_α-radiation (λ 1.5418). The intensities of the diffraction rings were estimated visually on an arbitrary scale of 1 to 10 and are quoted in parentheses: 8.85 Å ($\frac{1}{2}$), 5.77 (7), 5.17 (4), 4.41 (10), 4.26 (10), 3.88 (8), 3.84 (8), 3.76 ($\frac{1}{2}$), 3.58 (5), 3.48 ($\frac{1}{2}$), 3.28 ($\frac{1}{2}$), 2.91 (1), 2.87 ($\frac{1}{2}$), 2.71 (3), 2.63 (2), 2.47 (3), 2.42 (1), 2.22 (3).

Diphenylphosphorylethanolamine Hydrobromide

Through a solution of 3.0 gm. of O-diphenylphosphoryl carbobenzoxyethanolamine in 20 ml. of glacial acetic acid was passed a stream of hydrogen bromide† gas for one-half hour. The solution was occasionally cooled. After the solution was left for one-half hour at room temperature, the solvent and excess of hydrobromide were removed under reduced pressure (8–10 mm. Hg) at a bath temperature of 35°–40°. To the solution of the residual oil in 5 ml. of 99% ethanol, ether was added until the solution was permanently turbid, and the mixture was placed in an ice-box overnight. The crystalline diphenylphosphorylethanolamine hydrobromide was collected with suction on a buchner funnel and was dried *in vacuo* over paraffin and calcium chloride. Yield 2.32 gm. (88% of theory), m.p. 115°–116.5° (with slight sintering at 114°). $C_{14}H_{17}O_4NPBr$ (374.2). Calculated: C, 44.93; H, 4.58; N, 3.74; P, 8.28. Found:‡ C, 44.96, 45.27; H, 4.75, 4.65; N, 3.79; P, 8.32.

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**All melting points were determined in capillary tubes using an electrically heated bath of n-butyl phthalate and short-stem thermometers with a range of 50°.*

†The gaseous hydrogen bromide was prepared by the action of bromine on tetrahydronaphthalene.

‡Analyses of two independent preparations.

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A SEARCH FOR THE CHEMICAL EFFECTS OF INTERNAL CONVERSION FOLLOWING RADIATIVE NEUTRON CAPTURE[1]

By A. G. Maddock and M. M. de Maine[2]

ABSTRACT

The possibility that neutron capture would lead to oxidation, because of internal conversion of the capture radiation, has been investigated in solid compounds of the lower valence states of antimony, arsenic, cerium, chromium, and thallium. Except in the hydrated compounds, negative results have been obtained. It is possible that oxidation is followed by electron-transfer exchange in the irradiated solids.

INTRODUCTION

When an atom captures a thermal neutron, there results a highly excited compound nucleus which quickly radiates its excess energy in one or more gamma-rays. Conservation of momentum requires that the emission of these gamma-rays imparts to the newly formed atom a recoil energy many times that required for breaking chemical bonds. It is also possible that one or more of the gamma-rays might be internally converted and in this instance, oxidation of the atom will result. Most studies on the chemical changes following thermal neutron capture have focused on the bond breaking mechanisms, but there could be oxidation effects due to internal conversion, even if simple ionic solids were irradiated (10).

The physical detection of internal conversion following radiative neutron capture is attended with some experimental difficulties; but with a 180° beta-ray spectrograph Hibdon and Muehlhause (7) have detected the conversion electrons from neutron capture in $_{48}Cd^{113}$, $_{66}Dy^{164}$, $_{64}Gd^{odd}$, $_{88}Hg^{199}$, and $_{62}Sm^{149}$. Thermal neutron irradiation of ethyl bromide and iodide at pressures of three microns leads to 12 to 50% of the radioactive atoms of the different isotopes becoming positively charged as a result of internal conversion (16). Most of the gold and iridium recoil atoms which escape from the surface of gold and iridium films during neutron irradiation are positively charged (12, 19).

It seemed possible that chemical methods might provide a more sensitive means of detection and estimation of the internal conversion. Radioactive perrhenate has been separated after dissolution of neutron-irradiated rhenium trichloride (6). Following the neutron irradiation of several hydrated chromic salts, 3 to 10% of the radioactive chromium atoms were found in the hexavalent state upon solution and analysis of the crystals (4).

EXPERIMENTAL

In the present work, neutron irradiation of several simple inorganic salts with the cation in the lower of its two stable chemical valences was followed by dissolution of the solids in solutions containing as carriers cations of both valence states. There followed a chemical separation of the cations by a method

[1]*Manuscript received December 12, 1955.*
Contribution from the University Chemical Laboratory, Pembroke Street, Cambridge, England.
[2]*Lately Miss M. M. Moodie.*

which was chosen to avoid exchange between the two valence states. Measurements of the fraction of the radioactivity associated with each of the valence states were made. It was hoped that these would indicate the fraction of events leading to internal conversion and a consequent valence increase. Table I gives the present results together with those of Harbottle (4) for the hydrated chromic salts.

TABLE I

PERCENTAGE VALUES OF ATOMS PASSING INTO HIGHER VALENCE STATES DURING THE CONTROL ANALYSIS AND AFTER THERMAL NEUTRON IRRADIATION AND ANALYSIS
The values denoted* are those obtained by Harbottle (4)

Compound irradiated	Percentage of atoms in higher valence after irradiation and analysis		Percentage of atoms passing into higher valence during control analysis
Sb_2O_3	Sb^{122}	0.4	0.6
	Sb^{124}	0.4	
$KSbC_4H_4O_7.\frac{1}{2}H_2O$	Sb^{122}	1.8	
	Sb^{124}	1.8	
As_2O_3	1.0		1.1
$Ce_2(SO_4)_3$	Ce^{141}	2.0	2.0
	Ce^{143}	2.0	
$CrCl_3.10H_2O$	1.1		
$Cr_2(SO_4)_3.18H_2O$	2.9		1.2
$Cr_2(SO_4)_3.18H_2O$*	3.7		
$Cr(NO_3)_3.9H_2O$*	7.6		
$Cr_2(SO_4)_3.K_2SO_4.24H_2O$*	8.4		
$Cr_2(SO_4)_3(NH_4)_2SO_4.24H_2O$*	9.8		
Tl_2CO_3	0.9		
$TlNO_3$	0.9		0.9
Tl_2SO_4	0.9		

The methods of analysis for compounds of the different cations are as follows:

Antimony.—A magnesium chloride – hydrochloric acid solution of the irradiated compound with inactive Sb^{+3} and Sb^{+5} carriers was shaken with isopropyl ether which extracted the pentavalent antimony (2).

Arsenic.—The arsenate was separated from arsenite by precipitation of magnesium ammonium arsenite according to the procedures of Wilson and Dickinson (18).

Cerium.—Ceric compounds were extracted from cerous compounds by the use of ether after solution in nitric acid (8).

Chromium.—In one aliquot chromate was precipitated on the addition of lead nitrate solution. A second aliquot was treated with sodium hydroxide – sodium peroxide to oxidize chromic ions to chromate, and the lead chromate was then precipitated (3).

Thallium.—Thallous chromate was precipitated from the first aliquot. Sodium bisulphite treatment of a second aliquot reduced thallic ions to thallous, and the total thallium was precipitated as thallous chromate (5, 14).

DISCUSSION

Negative results were obtained except where hydrated compounds were used. In these cases very strongly oxidizing species might be formed by the action of the ionizing radiations on the water. The same remarks apply to aqueous solutions in which oxidizing effects have also been revealed (13). Positive effects in anhydrous compounds are necessary if it is to be argued that internal conversion is responsible.

Unfortunately these negative results do not prove that internal conversion does not take place. Indeed, the physical investigations (7) suggest that it is sufficiently commonplace to have occurred in one or more of these isotopes. The detection method used is only valid if exchange by electron transfer does not take place in the solid or during dissolution. The control tests only show that it does not interfere after dissolution.

Very little evidence is available concerning exchange in solid systems. The phenomenon of induced exchange during precipitation is widely recognized (15), but it has not been investigated systematically. In fact, it is difficult to obtain reproducibility in such measurements. Nevertheless, the existence of this phenomenon suggests that exchange might take place during the solution of solids containing atoms in two valence states.

In a number of solids, containing different valences of one element, physical evidence such as the optical and electrical properties suggests that some delocalization of valence electrons takes place. Such a process might be expected to lead to exchange by electron transfer. In some cases a similar interaction of atoms in two valence states can occur in solution (9, 17).

However, exchange is not inevitable even when interaction between the atoms seems to occur. Thus the deep orange lead sesquioxide can be prepared and decomposed without exchange taking place (20). Similar results have been obtained with chromic chromate (1, 11). This latter result suggests that exchange need not occur, at least in oxygenated chromic compounds. Such salts are all hydrated, however.

Even the absence of exchange during precipitation and subsequent dissolution of the compounds studied does not preclude electron transfer within the solid lattice. The radioactive atom suffers a considerable recoil and frequently settles down in some interstitial or other abnormal lattice position. Nothing is known about the probability of electron transfer involving such atoms, but it is probably enhanced.

The previous observation on rhenium trichloride (6) seems all the more surprising in the light of these results.

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INFLUENCE OF METHANOL ON VISCOSITY AND LIGHT SCATTERING PROPERTIES OF DEXTRAN SOLUTIONS[1]

By W. Donald Graham, Odette Patry, and E. Helen Jackman

ABSTRACT

Failure to consider the presence of up to 16% by volume of methanol in solutions of dextran fractions had a very marked effect on *apparent* intrinsic viscosity determinations (the term *apparent* signifies that measurements were made assuming that the solvent was water only). Unless methanol were removed or otherwise taken into account, high erroneous results were obtained. *Apparent* weight average molecular weights determined by light scattering were not significantly affected at these alcohol concentrations. The relations found over the range 0 to 16% methanol for dextran samples with weight average molecular weights of 265,000, 155,000, and 72,000 held for the latter sample up to 44% methanol. In the higher range of alcohol concentration the *apparent* weight average molecular weight was depressed. The true intrinsic viscosity of dextran solutions decreased as methanol concentration was increased.

During the course of testing dextran plasma expanders for conformity with various specifications, fractions representing the upper and lower 5 to 10% of the molecular weight distribution of the polymer are separated, usually by fractional precipitation with methanol. In some procedures no definite recommendation is made to ensure removal of traces of methanol which may remain with the precipitated dextran. According to Wolff *et al.* (8) this sirupy residue may contain up to 15% of methanol. Where great care in the separation of the supernatant solution from the viscous dextran-bearing precipitate is not exercised, it may be appreciated that variable and quite significant amounts of methanol may find their way into the test solution. In practice, when sharp separation of the layers is recognized as essential, the concentration of methanol in the final test solution (approximately 1 to 2% dextran in 100 ml. of solution) would be unlikely to exceed 2%. If test fractions are freeze-dried before they are dissolved for viscosity determinations etc., the methanol content should be of no practical significance.

Some laboratories have used light scattering measurements to assess the weight average molecular weights of these high and low fractions directly: others have made intrinsic or inherent viscosity determinations and converted the values thus obtained to "weight average molecular weights" by the application of a mathematical relationship (2, 3, 4, 6). The question arose as to the possible effects of varying amounts of methanol in the solutions on the subsequent molecular weight determinations. In an attempt to answer this question the present investigation was undertaken.

It was considered that the presence of methanol might have a greater effect on high molecular weight fractions than on low molecular weight material since larger dextran molecules tend to precipitate at lower concentra-

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Contribution from the Food and Drug Laboratories, Department of National Health and Welfare, Ottawa, Canada.

tions of methanol. Accordingly three dextrans with weight average molecular weights of approximately 265,000, 155,000, and 72,000 respectively were selected for this study.

EXPERIMENTAL AND RESULTS

Approximately 2.0% solutions of these dextrans were prepared in essentially dust-free, glass-distilled water and to 50 ml. aliquots of these solutions, varying amounts of methanol (B.P.C. grade, absolute) from 0 through 16 ml. were added. Sufficient water was added to bring each solution to 100 ml. These solutions were intended to represent dextran fractions which were obtained by methanol precipitation and in which varying amounts of contaminating methanol were present. From each of these solutions four dilutions were prepared in essentially dust-free water to contain approximately 0.15, 0.2, 0.25, and 0.35 gm. of dextran/50 ml. These dilutions were clarified by filtration through an ultrafine, sintered glass, pressure filter so that the dissymmetry of the filtered solutions in the Brice-Phoenix light scattering photometer, series 1000, was not greater than 1.15 at λ 436 mμ. It was noted that filtration became progressively slower as the amount of methanol present increased and the filters required frequent cleaning with hot nitric and sulphuric acid (1:1). The absolute turbidities of the solutions were measured and the solutions were reserved for polarimetric and viscosity measurements. Turbidities were corrected for the turbidity of the solvent* and the expression Hc/τ† was calculated for each dilution. From the Hc/τ vs. c data the intercept at $c = 0$ was calculated by least squares and the reciprocal of this intercept was recorded as the *apparent* weight average molecular weight.

No attempt was made to allow for the fact that measurements were made in a three-component system as no such correction would be made by the analyst who ignored or was unaware of the presence of methanol in his solutions. For the same reason, in the determination of the viscosities of these solutions as described below, the solvent was considered to be water rather than water–methanol mixtures.

The viscosities of the four dilutions were determined using Ostwald-Cannon-Fenske viscosity pipettes (1) series 50, all of which had flow times with water at 25°C. in excess of 240 sec. Duplicate determinations were required to check within 0.3 sec. The viscosity data were converted to *apparent* relative viscosities and thence to *apparent* specific viscosities (*app.* η_{sp}). The *app.* η_{sp}/c vs. c data were utilized in the least squares calculation of the intercept at $c = 0$ which was recorded as *apparent* intrinsic viscosity (*app.* $[\eta]$). Concentrations of the solutions under test were determined polarimetrically in a Rudolph precision photoelectric polarimeter assuming $[\alpha]_D^{20} = +200°$. The effect of methanol was ignored since it has been shown by Snyder *et al.* (5) that the specific rotation is increased by only about 1% by the presence of 40% methanol by volume. The data arising from the viscosity and light scattering measurements are recorded graphically in Fig. 1.

*Turbidities of the aqueous methanol solutions were not greatly different from that of water which commonly was 4 to 5 × 10^{-5} cm.$^{-1}$.

†$H = 0.624 \times 10^{-5}$, c = concentration in gm./ml., τ = turbidity in cm.$^{-1}$.

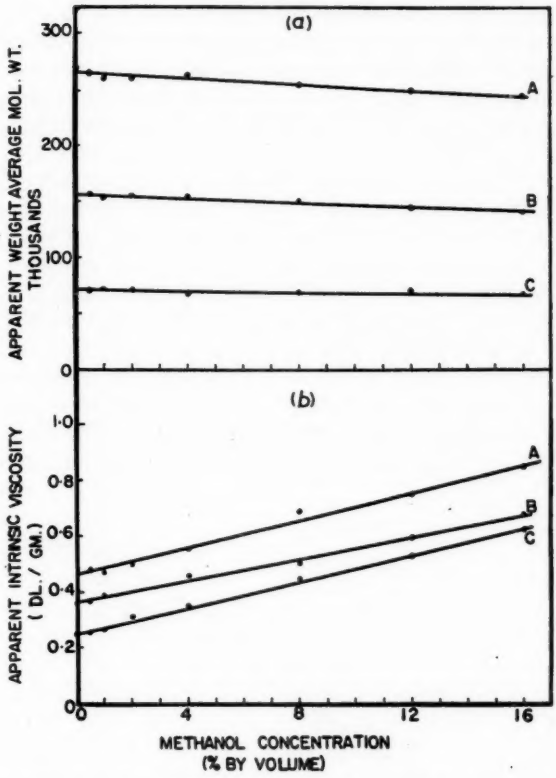

FIG. 1. Effect of methanol on (*a*) light scattering and (*b*) viscosity measurements on dextran solutions. Curve A—dextran sample 1, wt. av. mol. wt. 72,000; Curve B—dextran sample 2, wt. av. mol. wt. 155,000; Curve C—dextran sample 3, wt. av. mol. wt. 265,000.

It is obvious that the presence of amounts of methanol up to 16% by volume in the original dextran solution had relatively little effect on the *apparent* weight average molecular weight as determined by light scattering. The slight decrease in *apparent* weight average molecular weight with increasing methanol concentration is within the limits of reproducibility of the method (2) although the consistent trend suggests (*a*) increasingly serious loss of the larger molecules with increasing methanol concentration during filtration, (*b*) an error associated with ignoring the effects of the third component in the system, or (*c*) a combination of (*a*) and (*b*). Support for the first of these is found in the increasingly difficult filtration with increasing methanol concentration, particularly with the higher molecular weight dextran. The effect of the methanol *per se* might be expected to be small because the refractive index for aqueous methanol solutions in this concentration range is close to that of water (7). The gradual decrease in apparent molecular weight was associated with a small decrease in the second virial coefficients as the methanol concentration increased.

Light scattering measurements on sample 3 were extended up to methanol concentrations of 44%. The initial trend, apparent in Fig. 1(*a*) curve C, was continued and, at a methanol concentration of 44% in the starting dextran solution, the *apparent* weight average molecular weight was further depressed to about 64,000 from the initial 72,000 measured in water. This would appear to be a significant degree of depression (2).

In the case of *apparent* intrinsic viscosity, Fig. 1(*b*), the values obtained ignoring the presence of methanol rose very steeply as the methanol concentration increased. While it is unlikely that methanol concentrations in the dextran fraction solutions, as obtained in practice, would reach 16%, measurements on sample 3 were extended up to the point of incipient precipitation at 44% methanol. The curve continued upward with the same trend shown in the lower ranges in curve C, Fig. 1(*b*).

In Fig. 2 have been plotted (curve A) the *apparent* relative viscosities of solutions containing 0.5% of dextran sample 3 and increasing amounts of methanol. The *apparent* relative viscosities were obtained by dividing

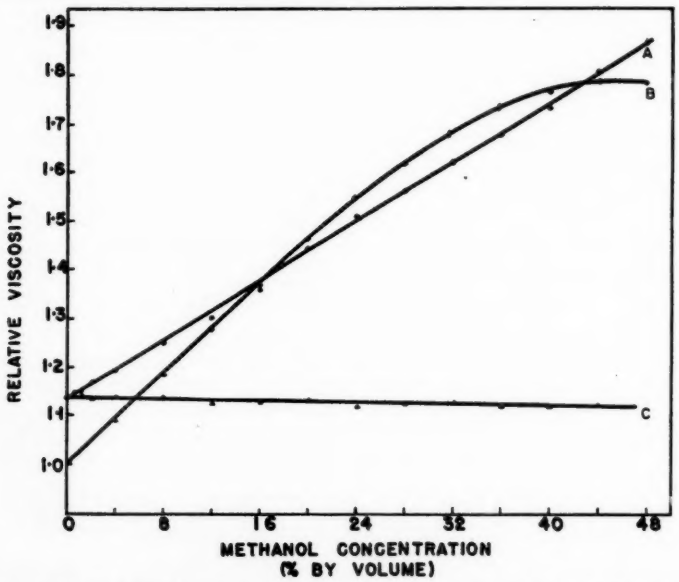

FIG. 2. Relative viscosities. Curve A—*Apparent* relative viscosity of a 0.5% solution of dextran sample 3 in the presence of increasing amounts of methanol. See text. Curve B—Relative viscosity of methanol solutions 0 to 48% by volume at 25°C. Curve C—Relative viscosities of solutions containing 0.5% of dextran sample 3 and varying amounts of methanol up to 44% by volume.

$\eta_{solution}$ by η_{water} ignoring the presence of methanol. The correction applied for density of the solutions was that applicable to dextran in water. In the same figure are plotted the true relative viscosities for aqueous methanol solutions with and without dextran (0.5% of sample 3) over the same range of methanol

concentrations (curves C and B respectively). Since the viscosities of water–methanol mixtures show a maximum for intermediate compositions, the increase in viscosity with increasing methanol content can be attributed to the solvent viscosity.

When appropriate aqueous methanol solutions are used to determine $\eta_{solvent}$, and the true $\eta_{relative}$ for each dextran dilution is determined, the η_{sp}/c may be calculated for each dilution. From these figures, by least squares extrapolation to $c = 0$, a value related to intrinsic viscosity may be obtained. Starting with the usual 1% dextran solutions containing increasing amounts of methanol up to 44% by volume, curve A shown in Fig. 3 was obtained for sample 3. Corrections for density were applied in the viscosity determinations.

It was of interest to see what deviation existed between curve A, Fig. 3, and the true intrinsic viscosity vs. methanol concentration curve. The necessary determinations were made with the four dextran dilutions used in obtaining $[\eta]$ at a specific methanol concentration, each containing that concentration of methanol. The curve B, resulting from these determinations, is shown in Fig. 3. As might be expected, curves A and B are somewhat similar.

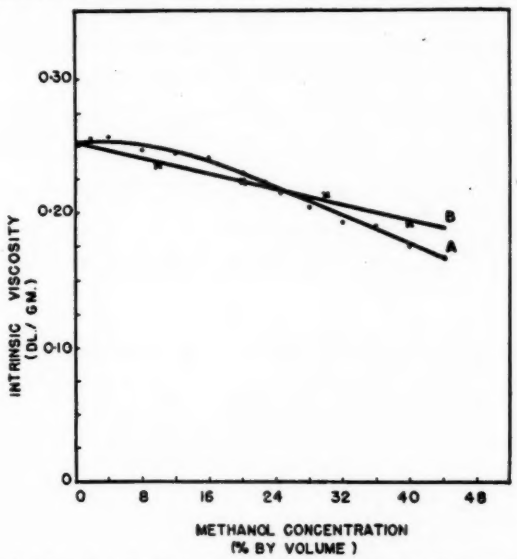

FIG. 3. Intrinsic viscosity of solutions of dextran sample 3 in the presence of 0 to 44% methanol by volume. Curve A—Pseudo intrinsic viscosity vs. methanol concentration. See text. Curve B—True intrinsic viscosity vs. methanol concentration.

Wales and co-workers have shown (7) that the $[\eta]$ of a similar dextran in 40.5% methanol in water was 0.17 as compared with 0.271 in aqueous solution. The results in the present instance are of the same general order. From curve B, which is directly comparable, the figures are, respectively, 0.193 and 0.253. From curve A, which is not directly comparable, the figures are 0.175 and 0.253.

The common purpose of performing viscosity determinations in the production of dextran plasma expanders is to control the molecular size of the product. The intrinsic or inherent viscosities so measured may be converted to a calculated viscosity average molecular weight for ease of comparison with other materials. If the formula derived by Wales and co-workers (6) for the relation between intrinsic viscosity and weight average molecular weight be used, the unknown presence of 2% of methanol in the test fraction for sample 3 will cause a more than 30% error in the calculated molecular weight. With the use of other formulae (2, 3, 8) the discrepancy will be even greater. The effect of 2% methanol on the light scattering weight average molecular weight is virtually nil. This example serves to emphasize the necessity for exercising great care in viscosity determinations on dextran fractions obtained by methanol precipitation.

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LEAD TETRAACETATE OXIDATION OF OLIGOSACCHARIDES[1]

By A. S. Perlin and A. R. Lansdown[2]

ABSTRACT

Lead tetraacetate oxidation is shown to be useful for examining some structural features of trisaccharides and higher oligosaccharides. Generally, the oxidations follow a course similar to that found with the corresponding disaccharide. In some oligosaccharides, however, glycol groups which resist oxidation are encountered. Such groups are 2,3-*trans* glycols of central residues which are linked to adjacent residues by 1,4-glycosidic bonds. The apparent inability to complex with lead tetraacetate suggests a conformation for these sugar residues in acetic acid solution which differs from that of the corresponding monosaccharide glycopyranoside. Lead tetraacetate oxidation of oligosaccharides in which central residues are unaffected by the reagent constitutes a unique stepwise degradation of these compounds, and possible applications of the reaction are suggested.

An earlier publication (11) described the use of lead tetraacetate oxidation (1, 4) for determining the structure of reducing disaccharides, and suggested that the reaction may also be of value for higher oligosaccharides. Subsequent examination of several oligosaccharides supports this contention.

In some respects the oligosaccharides show oxidation behavior which is closely similar to that of the disaccharides. Thus the yield and rate of production of formic acid from xylotriose and higher oligosaccharides of the same series are almost identical with those from xylobiose (11, Fig. 4). The rapid liberation of one mole of acid appears to result from cleavage of carbon–

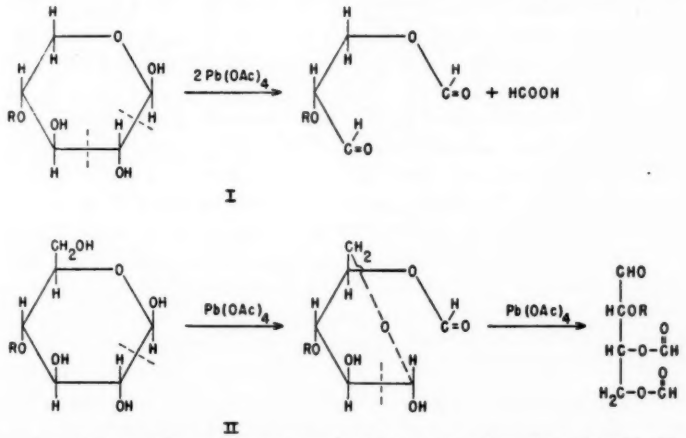

Fig. 1. I. Oxidation of the reducing end-unit of a (1 → 4) xylose oligosaccharide (R = non-reducing residue or residues). II. Oxidation of the reducing end-unit of a (1 → 4) glucose oligosaccharide (R = non-reducing residue or residues).

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[2]*National Research Council of Canada Postdoctorate Fellow, 1953–55.*

carbon bonds 1,2 and 2,3 of the reducing end-unit, carbon-1 being converted to a formate ester (I, Fig. 1) and carbon-2 released as formic acid; the second mole of acid is produced slowly from the non-reducing end. With oligosaccharides of the maltose and cellobiose series the formic acid produced appears to originate almost exclusively from the non-reducing end and, in each instance, at a rate approximately equal to that found for maltose (11, Fig. 1). In these compounds it appears that carbon-2 as well as carbon-1 of the reducing end-unit produces a stable formate ester (II, Fig. 1), and a total yield of only one mole of free acid is obtained.

From the standpoint of formic acid production the oligosaccharides considered above may be regarded as disaccharides since the central residues are glycosidically linked 1,4- and can yield no acid. However, it would be expected that the free 2,3-glycol group of the central residues should cleave oxidatively and lead tetraacetate consumption accordingly increase by one mole for each central unit. That is, xylobiose consumes four moles of oxidant but xylotriose should consume five moles, xylotetraose, six moles, and so on. In fact, however, all three compounds consume approximately four moles of lead tetraacetate per mole during the reaction period examined (Table I). Since this quantity is

TABLE I

LEAD TETRAACETATE OXIDATION OF OLIGOSACCHARIDES*

Compound	Total $Pb(OAc)_4$ consumed†	Formic acid produced	$Pb(OAc)_4$ consumed by compound
Xylobiose	5.8	1.9	3.9
Xylotriose	5.9	2.0	3.9
Xylotetraose	5.3	1.8	3.5
Xylopentaose	5.6	1.9	3.7
Xylohexaose	6.5	2.1	4.4
Maltose	5.2	1.1	4.1
Maltotriose	5.4	1.2	4.2
Maltotetraose	3.9	0.7	3.2
Maltopentaose	5.0	0.9	4.1
Maltohexaose	5.7	0.9	4.8
Cellobiose	4.9	1.1	3.8
Cellotriose	4.5	1.0	3.5
Cellotetraose	5.1	1.1	4.0
Benzyl β-maltoside	3.1	1.0	2.1
Raffinose	6.7	1.9	4.8

*Six-hour reaction period (see Fig. 1).
†Includes $Pb(OAc)_4$ consumed in oxidation of formic acid to CO_2.

sufficient only to account for the two moles of formic acid produced it appears that the central residues are not attacked by the oxidant. In agreement with this suggestion, after the oxidation of xylotriose one mole of pentose remains intact. Similar results are obtained with compounds of the maltose and cellobiose series (Table I). Approximately four moles of lead tetraacetate are consumed which again corresponds to oxidation only of reducing and non-reducing end-units. In a few instances, the values are only 80–85% of those generally found which is probably due to the fact that these compounds are

difficult to purify or are highly hygroscopic. Maltohexaose appears to consume an additional mole of oxidant per mole, suggesting that one of the four central residues is attacked.

Benzyl β-D-maltoside also exhibits a similar resistance to oxidation by lead tetraacetate. One mole of formic acid is liberated at a rate close to that of the methyl glucoside, and two moles of oxidant are consumed. These data account only for oxidation of the terminal unit, and one mole of glucose is recovered after hydrolysis of the reaction product.

The resistant sugar residues in these oligosaccharides have one common structural feature—a 2,3-*trans* glycol group subtended by glycosidic linkages at carbons 1 and 4. Lead tetraacetate is known to cleave *trans* glycol groups although less readily than *cis* glycols (4, 7). This has been explained in part by assuming that the cleavage reaction involves formation of a cyclic intermediate (4) which is more easily accommodated by a *cis* than by a *trans* glycol group. Accordingly, the configuration of the 2,3-*trans* glycol of the resistant units of the oligosaccharides may be distorted sufficiently to prevent complex formation. The 2,3-*trans* glycol group of the non-reducing end-unit, however, is readily oxidized and must therefore possess a more favored configuration.

Raffinose affords an oligosaccharide in which the central unit possesses a 2,3-*trans* glycol group but is linked through the 6- rather than the 4-position to an adjacent residue. If the latter linkage position is important for inducing resistance to oxidation, normal attack of the central unit in raffinose would be expected. This, in fact, is found to occur; two moles of formic acid are liberated and a total of five moles of lead tetraacetate consumed (Table I), corresponding to cleavage of all glycol groups in the molecule.

It appears, therefore, that the presence of a glycosidic bond at carbon-4 of a non-reducing sugar unit markedly alters the configuration of the 2- and 3-hydroxyl groups from that normally encountered in the corresponding monomer glycopyranoside. A critical evaluation of glycol-cleavage oxidations by Heidt, Gladding, and Purves (7) led to the assignment of optimal dimensions in glycol groups for complexing with an oxidant. Studies by Reeves on cuprammonium complexes of glycosides likewise suggest conformations which are likely to be less or more prone to glycol-cleavage (12). From these data, the observed resistance of glycol groups in oligosaccharides to oxidation suggests that the sugar units concerned may possess a conformation in acetic acid solution other than Reeves' C-1 form, most commonly encountered with the glycopyranosides of these sugars (12). Steric hindrance to complexing of the glycol group with the oxidant by neighboring sugar residues appears unlikely because differences in the degree of resistance should then have been expected between α- and β-linked compounds, e.g. between maltotriose and cellotriose. It will be of interest to examine other oligosaccharides which have structural features somewhat different from those described above. For example, a trisaccharide in which mannose constitutes a central residue linked 1,4- to the adjacent units would provide a 2,3-*cis* glycol group which would be expected to cleave readily. Considerations of this kind have been recorded

by Dimler (5) in explaining the resistance of ⟨1,4⟩⟨1,6⟩ anhydrohexofuranoses to periodate and lead tetraacetate oxidation. In these hexosans the resistant glycol group is also *trans* but, in contrast to those of the oligosaccharides described, occurs on a furan ring and hence is more likely to have a highly unfavorable bond angle, i.e. about 120° or greater (12).

The current results show that lead tetraacetate oxidation under the conditions described may be of value for determining some structural features of higher oligosaccharides. Limitations are imposed, however, by the resistance to oxidation of some types of glycol groups. For example, distinction between a 1,3- and 1,4-glycosidic linkage in central sugar residues may prove difficult in such instances, requiring collaborative evidence from methylation or periodate oxidation. On the other hand, this behavior can facilitate the determination of the linkage position of the reducing end-unit, as indicated above, or the identification of the central residues of oligosaccharides containing more than one type of monosaccharide residue. The reaction also constitutes a unique means for effecting a partial degradation of many oligosaccharides. Application may therefore be found, for example, in studies with isotopically-labelled compounds. Thus, the specific activity of the central unit in maltotriose could be determined by treating the compound with lead tetraacetate and isolating by hydrolysis the unoxidized glucose. The observed instances of resistance to oxidation, however, question the practice of using lead tetraacetate to test for glycol groups in partially substituted polysaccharide derivatives. Polysaccharides examined, including glycogen and a water-soluble pentosan, did not consume lead tetraacetate during a reaction period of several days' duration. However, these instances of unreactivity may be attributed chiefly to insolubility of the carbohydrates in acetic acid, for otherwise, by analogy with the oligosaccharides, end-groups of the polymers should be oxidized.

EXPERIMENTAL

The oligosaccharides of the cellobiose, maltose, and xylobiose series were kindly furnished, respectively, by Dr. D. R. Whitaker, Dr. R. L. Whistler, and Dr. C. T. Bishop, who have described the compounds elsewhere (15, 14, 2). Benzyl β-maltoside was prepared by the procedure of Lemieux and Lansdown (8). Lead tetraacetate was prepared according to the procedure recommended by Vogel (13), or was obtained commercially from Matheson, Coleman, and Bell, New Jersey.

Oxidation of Oligosaccharides

Reactions were carried out in duplicate at 28°C. in a constant-volume type of Warburg respirometer as described previously (10, 11) using aqueous acetic acid as solvent (1) to facilitate solution of the carbohydrates. In a typical experiment, one milliliter of 90% acetic acid containing 20 mgm. of lead tetraacetate and 10 mgm. of potassium acetate was placed in the vessel, and 1.24 mgm. of xylotriose, dissolved in 0.2 ml. of 90% acetic acid, was placed in the side-arm. After initial equilibration the reaction was initiated by mixing the two solutions and changes in pressure due to evolved carbon

dioxide were noted at desired intervals, the readings being corrected for pressure changes in the respirometer blank. The rate of evolution of carbon dioxide is represented approximately by curve 2 (11, Fig. 4); in six hours' reaction time the rate had become slow and the yield of carbon dioxide corresponded to a value of 2.0 moles of formic acid per mole of xylotriose. The contents of the vessel were transferred to a glass-stoppered flask by washing with 10 ml. of "stopping" solution (3), and the quantity of lead tetraacetate consumed in the reaction was determined by titration with thiosulphate. The difference in titer between the blank and sample was 7.10 ml. of 0.005 *N* thiosulphate corresponding to 5.9 moles of oxidant consumed per mole of xylotriose. Since 2.0 moles of lead tetraacetate were used in converting the formic acid to carbon dioxide the corrected consumption of oxidant was 3.9 moles per mole (Table I).

In a duplicate experiment, at the end of the six-hour period the reaction mixture was treated with 20 mgm. of oxalic acid, dissolved in 0.2 ml. of glacial acetic acid; this simultaneously reduced excess lead tetraacetate and precipitated the divalent lead. An aliquot of the supernatant solution was then used for colorimetric determination of pentose with the orcinol reagent (9). The quantity of pentose formed using xylobiose, oxidized under the same conditions, as blank corresponded to 0.89 moles of xylose per mole of oxidized xylotriose.

The quantity of glucose remaining after oxidation of benzyl maltoside was determined as follows: after the treatment with excess oxalic acid, as above, the acetic acid was distilled and the residue was dissolved in water and heated at 100°C. for 3.5 hr. The hydrolyzate was then analyzed for glucose by quantitative paper chromatography (6); the glucose content of the solution corresponded to 0.96 mole per mole of benzyl maltoside.

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THE PAPILIONACEOUS ALKALOIDS

XXII. PUSILLINE; ITS IDENTITY WITH β-ISOSPARTEINE[1]

By R. GREENHALGH[2] AND LÉO MARION

ABSTRACT

l-Pusilline is shown to be identical with β-isosparteine by direct comparison of the bases and of their salts. By mild oxidation with mercuric acetate followed by catalytic hydrogenation, pusilline is converted to *d*-sparteine, thus confirming its identity with *l*-β-isosparteine. The sparteine obtained from β-isosparteine derived from lupanoline was *d*-sparteine, and not the *l*-form as reported. Oxidation of β-isosparteine with potassium permanganate gives rise to a hydrated 10,17-dioxo-derivative.

l-Pusilline, first isolated from *Lupinus pusillus*, was described as a completely saturated alkaloid yielding analytical figures in agreement with either $C_{15}H_{26}N_2$ or $C_{15}H_{28}N_2$ (3). Since an imino-methyl determination indicated the presence of one such group, the latter empirical formula was adopted (4). Because of the marked similarity between its properties and those of sparteine, pusilline was tentatively represented as 3-(N′-methyl-2′-piperidyl)-quinolizidine. Of the four possible racemates of this structure, one has been synthesized by Winterfeld, Wald, and Rink (9) and two racemates were synthesized by Šorm (8). These, however, were different from the alkaloid.

Reduction of lupanoline, an isomer of hydroxylupanine (5), with lithium aluminum hydride has been shown by Moore and Marion (6) to produce β-isosparteine which was isolated as a diperchlorate. Recently, Carmack, Douglas, Martin, and Suss (1) have established the identity of the alkaloid spartalupine occurring in *L. sericeus* with β-isosparteine and thus recorded the first natural occurrence of the alkaloid. Spartalupine and pusilline both give rise to monoperchlorates which have the same melting point. It has now been shown that pusilline monoperchlorate can be converted to a diperchlorate having the same melting point as β-isosparteine diperchlorate, and having an X-ray powder pattern identical with that of the latter. A comparison of the infrared absorption spectrum of β-isosparteine (see Fig. 3 in Ref. 2) with that of pusilline revealed that each peak appearing in one was present in the other. Furthermore, mild oxidation of *l*-pusilline with mercuric acetate followed by catalytic hydrogenation gave rise to *d*-sparteine identified by comparison of its infrared absorption spectrum with that of an authentic specimen, and also by comparison of its dipicrate with those of both *l*- and *d*-sparteine. This conversion had already been described as occurring with β-isosparteine[3] (6).

A complex picrate had been obtained from pusilline (4). It has now been

[1]*Manuscript received December 16, 1955.*
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[2]*National Research Council of Canada Post-doctorate Fellow.*
[3]*Through an error, Moore and Marion (6) reported that the β-isosparteine obtained from lupanoline gave rise in this conversion to l-sparteine. This should read d-sparteine. The dipicrate melted at 209° and the melting point was not depressed on admixture with d-sparteine dipicrate; it melted at 203–204° when mixed with l-sparteine dipicrate. It had* $[\alpha]_D$ *+22 (c = 0.09 in methanol). An authentic sample of d-sparteine dipicrate had* $[\alpha]_D$ *+24.5 (c = 0.092 in methanol).*

shown that pusilline also forms a dipicrate identical with that prepared from β-isosparteine by Dr. Carmack who kindly sent us a specimen. The previously reported analytical determination of an N-methyl group was spurious since repeated determinations with pusilline and pusilline monoperchlorate gave negative results so long as *freshly distilled* hydriodic acid was used.

It has been previously reported by Moore and Marion (6) that the oxidation of β-isosparteine with potassium ferricyanide gives rise to oxo-β-isosparteine ($C_{15}H_{24}ON_2$), which by mercuric acetate oxidation followed by catalytic hydrogenation is converted to 17-oxo-sparteine. If, on the other hand, pusilline was oxidized with potassium permanganate it gave rise to a dioxo-compound which was neutral, and therefore a dilactam. It gave analytical figures in agreement with the formula $C_{15}H_{24}O_3N_2$. Its infrared absorption spectrum showed strong absorption in the region indicative of lactam carbonyls, and also a band in the hydroxyl region. When this compound was distilled *in vacuo* and crystallized from hexane it yielded two forms of crystals which were separated by hand. The first was the hydrate of a dioxo-compound ($C_{15}H_{22}O_2N_2.H_2O$) and the second the anhydrous product. An attempt to hydrogenate the dioxo-hydrate in acid solution over Adams' catalyst at room temperature proved unsuccessful, and this would seem to indicate a 10,17-dioxo structure, particularly in view of the potassium ferricyanide oxidation product.[4]

It can therefore be concluded unequivocally that *l*-pusilline and *l*-β-isosparteine are identical.

EXPERIMENTAL

Pusilline isolated from *L. pusillus* (3) consisted of a colorless oil, b.p. 110–115° at 4 mm., $[\alpha]_D^{32}$ −15.3° (*c*, 2.3 in abs. ethanol). It formed a monoperchlorate, m.p. 216°; a dihydrochloride, m.p. 270–273°; a monohydrochloride hydrate, m.p. 88–90°, resolidified and melted again at 235.5–236.5°; a monohydriodide, m.p. 252.5°; and a complex picrate, m.p. 183.5–185.5° (4).

Conversion of l-Pusilline to d-Sparteine

A small quantity of pusilline in 5% aqueous acetic acid was oxidized with an excess of mercuric acetate according to the usual procedure (6), and the dehydro-base was hydrogenated over Adams' catalyst in acid solution. The base thus obtained was distilled *in vacuo*; its infrared absorption spectrum was superimposable on that of *d*-sparteine. The base formed a dipicrate, m.p. 207–208°, either alone or in admixture with an authentic sample of *d*-sparteine dipicrate. Calc. for $C_{15}H_{26}N_2.2C_6H_3O_7N_3$: C, 46.82; H, 4.66. Found: C, 46.91; H, 4.88%. In admixture with *l*-sparteine dipicrate it melted at 202–203°.

Pusilline Diperchlorate

A quantity of the monoperchlorate of pusilline was dissolved in methanol and the solution neutralized to Congo red with perchloric acid. On standing,

[4]*Dr. Carmack informs us that he has established that the derivative so obtained from β-isosparteine is 10,17-dioxo-β-isosparteine, by comparison with one of the dl-dioxo-intermediates obtained in Sorm and Keil's synthesis (7) of sparteine. A sample kindly supplied by Dr. Carmack did not depress the melting point of our product on admixture.*

the liquor slowly deposited the diperchlorate. It was recrystallized from boiling methanol from which it separated as small, stout, colorless prisms, m.p. 247–249° (dec.), when immersed at 195°. In admixture with authentic *l*-β-isosparteine diperchlorate (6), m.p. 246–248°. The X-ray powder patterns of the two diperchlorates were identical.

Pusilline Dipicrate

Freshly distilled pusilline (15 mgm.) was dissolved in methanol and added to a methanolic solution of picric acid (30 mgm.). The resulting liquor was concentrated on the steam-bath and allowed to cool. It deposited a crystalline picrate which, after recrystallization from methanol, melted at 132–133°. The melting point was not depressed by admixture with a sample of β-isosparteine dipicrate kindly supplied by Dr. M. Carmack.

Oxidation of Pusilline (β-Isosparteine)

A quantity of pusilline in aqueous medium was oxidized with finely powdered permanganate. The addition of permanganate was stopped when the color was retained by the solution for several hours. The precipitated manganese dioxide was filtered, washed with water, and then with chloroform. The filtrate was extracted repeatedly with chloroform and the extract evaporated to dryness. There was left a crystalline product, m.p. 163–165°, which distilled at 90–110° at 0.04 mm. to a hygroscopic, opaque glass. On recrystallizing from hexane this product gave two forms of crystals which were separated by hand. One form consisted of long colorless needles, m.p. 170–171°, which was not depressed in admixture with a sample of dioxo-spartalupine (dioxo-β-isosparteine) hydrate kindly supplied by Dr. M. Carmack. Calc. for $C_{15}H_{22}O_2N_2.H_2O$: C, 64.26; H, 8.63; N, 9.99. Found: C, 64.55; H, 8.52; N, 10.21%. The other forms of crystals consisted of very small clusters, m.p. 154–155°, which gave a large depression when mixed with the substance melting at 170–171°. The analysis of this substance was not too satisfactory. Whereas the hydrogen and oxygen results showed good agreement with the figures required for the anhydrous compound, carbon was somewhat high.

ACKNOWLEDGMENTS

We wish to acknowledge our indebtedness to Dr. Maria Przybylska for the X-ray powder patterns and to Mr. R. Lauzon for the infrared spectra; also to Dr. Marvin Carmack for his courtesy in giving us samples of β-isosparteine dipicrate and dioxo-β-isosparteine hydrate.

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COMPOSITION OF DELPHINIUM SEED OIL[1]

By Mary J. Chisholm and C. Y. Hopkins

ABSTRACT

The fatty oil of delphinium seed (*Delphinium hybridum* (Hort.)) was examined. Fresh seed gave an oil composed mainly of glycerides but having 2.8% of free fatty acids. The oil from older seed contained about 50% of free fatty acids, apparently as a result of lipase action in the seed. The total fatty acids were found to include *cis*-11-eicosenoic acid (18%) and eicosadienoic acid (1%), the latter identified as tetrahydroxyeicosanoic acid. Other acids that were identified and the estimated percentages were: 9-hexadecenoic <1, palmitic 4, linoleic 16, oleic 53, and stearic 1. Spectroscopic analysis indicated a content of 2.5% of octadecatrienoic acid. Eicosoic acids have not been observed previously in the seed oils of this plant family (Ranunculaceae).

INTRODUCTION

The seeds of various plants of the Ranunculaceae have been observed to contain from 20 to 40% of fatty oil and to have a high lipase activity. Little is known of the composition of the oils. The constants of oils from *Delphinium* species have been recorded by Markwood (9, 10), Ivanov (8), and Hunter (7). One species, *Delphinium staphisagria*, has been studied further by Nath (11) who identified several of the fatty acids of the oil.

In the present work, the fatty acids of *Delphinium hybridum* (Hort.) have been studied in a quantitative manner and some attention has been given to the action of the lipase.

EXPERIMENTAL

Properties of the Oil

The seed examined in the main part of the work (Sample 1) was that of the cultivated delphinium, described as *D. hybridum* (Hort.). It was obtained from a commercial seed house and remained in storage for four years under dry conditions before the oil was extracted. On extraction with petroleum ether the seed yielded 36.1% of oil (basis 10% moisture). Constants of the oil are shown in Table I.

TABLE I

Properties of oil of *D. hybridum* (Hort.), Sample 1

Iodine value	101	Glycerol yield, %	5.0
Saponification value	194	Acetyl value	24.8
Unsaponifiable matter, %	1.0	Acetyl value of methyl esters	6.5
Acid value	100	Peroxide value (iodide method)	3.5
Free acid as oleic, %	50.3		

The high content of free fatty acid, considered to be the result of lipase action in the seed, is accompanied by a low glycerol yield, since glycerol liberated in the seed is known to disappear rapidly. The acetyl value is unusually

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high, probably because of the presence of partially hydrolyzed glycerides. It is reduced considerably when the glycerides are converted to methyl esters.

Seed of another strain of *D. hybridum* (Sample 2) was examined within a few months of harvesting. It yielded 25.7% of oil having iodine value 123 and acetyl value 4.7. This oil gave a glycerol yield of 9.7%. The acid value was 5.5, corresponding to 2.8% of free fatty acid. Evidently lipolysis had progressed to only a slight extent.

Seed of *Delphinium ajacis* (Sample 3) had 39.9% oil of iodine value 95 and acid value 72.1, corresponding to 36.3% of free fatty acid. This seed was more than one year old.

A portion of Sample 1 was saponified and the total fatty acids were freed from unsaponifiable matter. Their ultraviolet absorption, before and after

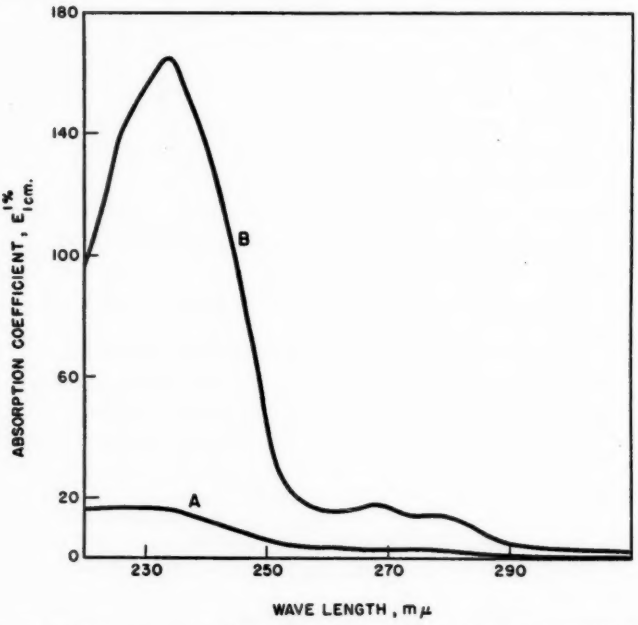

FIG. 1. Ultraviolet spectrum of delphinium fatty acids freed from unsaponifiable matter. *A*, before isomerization; *B*, after isomerization (in glycerol 45 min. at 180°).

alkali isomerization, is shown in Fig. 1. The absorption coefficients ($E^{1\%}_{1\,cm.}$) after isomerization were 164.5 at 233 mμ and 18.1 at 268 mμ, indicating a content of 15.9% of dienoic acid(s) and 2.5% of trienoic acid(s), calculated as octadecoic acids. The calculation is based on absorption coefficients for natural *cis* acids.

Examination of the Methyl Esters

The oil (Sample 1) was converted to methyl esters by methanolysis with acid catalyst (6). The esters (300 gm.) were distilled through a Podbelniak

Heli-Grid column at a pressure of 0.4 mm., and the residue was transferred to a smaller flask and distilled through a spinning band column. The distillation curve is shown in Fig. 2. It indicates an appreciable content of C_{20} esters. A further lot of esters was hydrogenated and distilled, giving a curve almost identical with the one for the original esters.

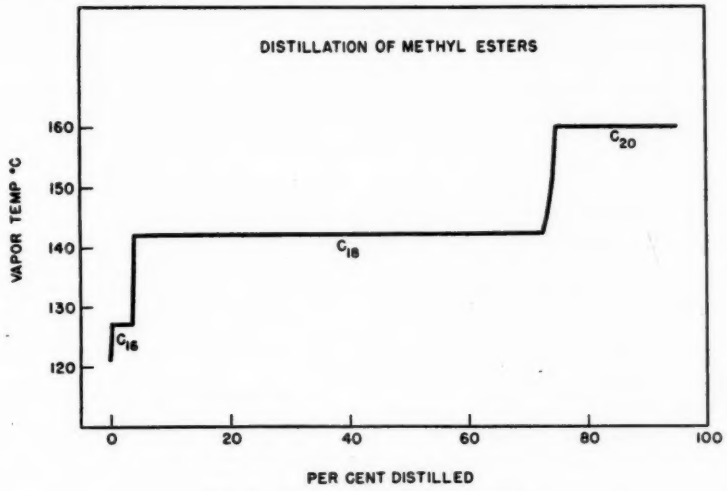

FIG. 2. Distillation of methyl esters.

Distillation data for one lot of the original methyl esters are given in Table II. The distilled fractions were examined individually by the methods

TABLE II

DISTILLATION OF METHYL ESTERS

Fraction	Temp., ° C. (0.4 mm.)	Weight, gm.	Chain length	Iodine value	Refractive index, 25°
1	120–127	1.1		20.3	—
2	127	10.0	C_{16}	6.4	Solid
3	142			116.7	1.4541
4	142	206.0	C_{18}	103.4	1.4528
5	142			95.1	1.4519
6	142–151	4.4		60.9	Solid
7	151–160	1.8		91.4	1.4585
8	160	26.7	C_{20}	81.2	1.4535
9	160	22.9	C_{20}	77.4	1.4529
R	-	17.0			

described previously (3) and the component acids were identified as shown in Table III. Identity of the products was confirmed by mixed melting point except in the case of tetrahydroxyeicosanoic acid, which has not been prepared previously.

TABLE III

IDENTIFICATION OF ACIDS

Fraction	Acid	Identified as:	Melting point, °C.
1	9-Hexadecenoic	9,10-Dihydroxypalmitic	123.5–124.5
2	Palmitic	Palmitic	62.5– 63
3	Linoleic	Tetrahydroxystearic	172 –173
	Oleic	Dihydroxystearic	129 –130
6	Stearic	Stearic	69 – 70
8	Eicosadienoic	Tetrahydroxyeicosanoic	172 –173
8	11-Eicosenoic	11,12-Dihydroxyeicosanoic	129.5–130.5
9	11-Eicosenoic	11-Eicosenoic N-hydroxyamide	69 – 70

Examination of C_{20} Fractions

Fractions 7 and 8 were found by ultraviolet absorption to contain some diene acid. Fraction 8 was recrystallized from acetone at −45°, giving 22.9 gm. of methyl eicosenoate, iodine value 77.9 (theory 78.2). After saponification and hydroxylation by permanganate, it yielded dihydroxyeicosanoic acid, m.p. 129.5–130.5°. The melting point was not depressed by mixing with authentic 11,12-dihydroxyeicosanoic acid.

The acetone filtrate from the recrystallization of Fraction 8 gave 2.8 gm. of methyl esters of iodine value 102.6, evidently containing the dienoic acid. After saponifying, hydroxylating with permanganate, and boiling the product with ethyl acetate to dissolve dihydroxy acids, the residue was recrystallized from ethanol. It melted at 172–173°. When mixed with tetrahydroxystearic acid of m.p. 172–173°, the melting point was lowered to 162–164°. Accordingly the substance is considered to be tetrahydroxyeicosanoic acid. Calc. for $C_{20}H_{40}O_6$: C, 63.79; H, 10.70. Found: C, 63.99; H, 10.74. Its infrared spectrum is consistent with this structure. The spectrum, determined in Nujol mull, is very similar to but not identical with that of 9,10,12,13-tetrahydroxystearic acid of m.p. 144° (R. N. Jones, to be published).

Fraction 9 was almost pure methyl eicosenoate. Its infrared spectrum showed no peak at 965 $cm.^{-1}$ and accordingly it is judged to be the *cis* form. The molar extinction coefficient ϵ at 965 $cm.^{-1}$ was 12.0 (methyl oleate 12.2).

The C_{20} fraction from the distillation of the hydrogenated esters was pure methyl arachidate, m.p. 45–46°. The small distillation residue was saponified. The acids had equivalent weight 318.9 (arachidic acid, 312.5), indicating that there is a small proportion of acid of greater chain length than C_{20}.

Percentage Composition

Distillation of the hydrogenated esters and examination of the residue gave the following percentages by chain length: C_{16} 5, C_{18} 70, C_{20} 19, C_{22} 1, loss and unidentified residue 5.

Estimation of the fatty acid composition from the entire data, including only those acids actually identified, gives the percentages shown in Table IV (in the order in which the esters distill).

TABLE IV

ESTIMATED FATTY ACID COMPOSITION (% OF TOTAL FATTY ACIDS)

Acid	%	Acid	%
9-Hexadecenoic	< 1	Stearic	1
Palmitic	4	Eicosadienoic	1
Linoleic	16	11-Eicosenoic	18
Oleic	53	Undetermined	6

The undetermined portion includes about 2.5% of trienes, as indicated by the spectrometric data, mainly in the C_{18} range. There was also some distillation loss and some unidentified material in the residue.

Glyceryl Residue and Lipase Activity

A sample of oil from seed sample 1 was saponified and the fatty acids were removed. The aqueous residue was concentrated and treated with benzoyl chloride. It yielded glycerol tribenzoate, m.p. 71–72° alone and mixed with an authentic sample. Oil from fresh seed was analyzed for glycerol by the method of Colson (4). It gave a glycerol yield of 9.7%.

The free fatty acids were extracted from the oil of Sample 1 and compared with the acids of the remaining glycerides to determine whether the enzyme acts preferentially with respect to type of acid. The free acids were 2 units lower in equivalent weight and 4 units lower in iodine value but it is doubtful whether these differences are sufficient to show any specificity.

There was no appreciable change in acid value during storage of the oil. Evidently the enzyme is destroyed or separated from the oil during the solvent extraction process.

DISCUSSION

The oil examined in this work is unusual in having a considerable content of eicosenoic acid (18%) but only a small amount of acids higher than C_{20}. Two studies of the composition of Ranunculaceae seed oils have been reported by earlier workers. *Nigella sativa* oil was found to contain palmitic, stearic, oleic, and linoleic acids (12). *Delphinium staphisagria* oil was reported by Nath (11) to contain oleic, elaidic, and linoleic acids. Thus there has been no previous record of C_{20} fatty acids in this plant family.

The eicosenoic acid isolated in the present work is the ordinary Δ^{11} isomer. It is now known to occur in appreciable amounts in one or more species of the following plant families: Crucifereae, Buxaceae, Tropaeolaceae, and Ranunculaceae.

The occurrence of eicosadienoic acid, $C_{20}H_{36}O_2$, in animal fats and phospholipids has already been established by several workers. It is considered to be of importance in nutrition. Some evidence of its presence in vegetable fats had been noted when determining unsaturation of C_{20} fractions by iodine values and ultraviolet absorption (1, 5). Until now, however, it does not appear to have been definitely identified as a constituent of a vegetable fat. The amount in the delphinium oil is about 1%.

D. ajacis seed was shown by Bamann and Ullmann to contain a highly active lipase which was more than twice as effective as *Ricinus* lipase (2). It is considered probable that this enzyme is responsible for the unusually high free fatty acid content found in the oil of *D. ajacis* in the present work. The oils of *D. hybridum* (present work), *D. consolida* (9), and *D. staphisagria* (10) have shown similar high acid values, equivalent to 45% or more of free fatty acids.

It may be pointed out that delphinium seed oil ordinarily contains a small percentage of alkaloids.

ACKNOWLEDGMENTS

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DECOMPOSITION OF SODIUM HYPOCHLORITE: THE UNCATALYZED REACTION[1]

By M. W. Lister

ABSTRACT

The decomposition of sodium hypochlorite has been re-examined. The results show that Foerster and Dolch's mechanism of the decomposition to chlorate and chloride is correct; they postulated a slow bimolecular reaction to chlorite and chloride, followed by a faster reaction of chlorite with more hypochlorite. Values of the rate constants of both steps are reported; they make the activation energies 24.8 kcal./gm-molecule for the first step and 20.8 kcal./gm-molecule for the second. The rates are such that at 40° C. a solution of sodium hypochlorite will contain about 1% as much chlorite as hypochlorite. The rate is strongly affected by changing ionic strength; at low ionic strengths it is nearly constant or falls slightly; above about 0.8, the rate rises and at high ionic strengths the rise is quite rapid. No signs of specific catalytic effects of sodium chloride, hydroxide, or carbonate could be observed, and it seems probable that earlier reports of this were due to variations in ionic strength. The decomposition to chloride and oxygen has been measured and is a unimolecular reaction, which is possibly, but not certainly, uncatalyzed. Values of its rate constant are reported; they also are much altered by changing the ionic strength.

Although this reaction was first investigated a considerable number of years ago, there are several matters connected with it that are still not entirely clear. Briefly, the position seems to be this. The best early work on the subject was that of Foerster and his co-workers, particularly Foerster and Dolch (2). They found it to be a second order reaction, and consequently deduced that the mechanism was:

$$2NaOCl \rightarrow NaClO_2 + NaCl$$
$$NaOCl + NaClO_2 \rightarrow NaClO_3 + NaCl\,.$$

The first step is the slower. They showed by an independent experiment that the second step was indeed faster, and their rate constants gave activation energies of $22\frac{1}{2}$ and $20\frac{1}{2}$ kcal./gm-molecule for the first and second step respectively. In addition to the reactions given above it has long been known that decomposition to sodium chloride and oxygen also occurs. Foerster and Dolch's rates are the total reaction rates, though they knew that the reaction to chlorate was very much the more important. They also found that the rate increased as they added sodium hydroxide. Later Giordani (3) investigated the reaction, and concluded that it was a combination of a termolecular reaction giving chlorate, and a bimolecular reaction giving oxygen. He also found a marked effect of sodium hydroxide, but in the opposite direction from that found by Foerster; and, at least from about 0.5 to 1.0 *M* sodium hydroxide, obtained rate constants proportional to $(NaOH)^{-2}$. He therefore assumed that hypochlorous acid was essential to the reaction, and that it went in one step, as follows:

$$OCl^- + 2HOCl \rightarrow ClO_3^- + 2HCl\,.$$

He proposed that the reaction to oxygen was

$$2OCl^- \rightarrow 2Cl^- + O_2\,.$$

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Contribution from the Department of Chemistry, University of Toronto, Toronto, Ontario.

Skrabal and Skrabal (6) examined the dependence of rate on pH, and agreed with Foerster (and Giordani) that the rate was second order at high pH. Pierron (5) examined the relative stability of lithium, sodium, and potassium hypochlorites. He seemed to favor Giordani's mechanism, but supposed that alkali metal peroxides were formed in the alkaline solutions, and that these promoted the reaction. Barredo (1) examined the reaction and found it to be second order; but he also found the rate to be altered by addition of chloride, and over a certain range the rate was proportional to the chloride concentration. He believed that this explained Giordani's results. Such a dependence on chloride concentration would of course make the reaction autocatalytic. The present author (4) has examined the rate of decomposition of neutral or slightly acid hypochlorite solutions, and found that under these conditions the main decomposition was of hypochlorous acid molecules, by a second order reaction. It can be calculated from these results (combined with those in the present paper) that once quite a small excess of sodium hydroxide is present, the concentration of hypochlorous acid is so slow as to make only a negligible contribution to the total decomposition.

It seemed possible that these reported effects of sodium hydroxide and chloride might really be due to changes in the ionic strength. In earlier work the ionic strength was not always kept constant from run to run; and it is not always possible in re-examining the data to deduce what the ionic strength really was. Accordingly the effect of ionic strength on the rate of reaction was examined in the present work. Foerster and Dolch's results seemed to establish that the reaction was second order; however, in view of contradictory later results, this point was checked.

Sodium hypochlorite decomposes both to chlorate and chloride, and to oxygen and chloride. The relation between these reactions was by no means clear, and the oxygen evolution had been relatively little investigated. Hence in the present work the two reactions were measured, so that their kinetics could be determined separately. Since the reaction to oxygen is strongly catalyzed by certain metallic oxides, notably copper or nickel oxides, there has always been some doubt that there really is any appreciable uncatalyzed evolution of oxygen. Hence in the present work, an attempt was made to be sure that the oxygen evolution was not catalyzed; and to find the order of this reaction, and the effect on the rate of varying temperatures or ionic strength.

MATERIALS AND EXPERIMENTAL METHODS

Sodium Hypochlorite

This was produced in solution by the usual method of passing chlorine through cold sodium hydroxide solution. The product always contained a small excess of sodium hydroxide to avoid hydrolysis to hypochlorous acid. The chief uncertainty with these solutions was that they might contain traces of metallic oxide which could act as a catalyst. Such impurities could easily be present in any commercial sample of sodium hydroxide to an extent that would be undesirable, and it would be very difficult to measure how much

was present accurately enough to allow for its catalytic effect. It was found however that if nickel or copper salts were added to the hypochlorite solution, they could be removed by the following procedure. A small amount of calcium chloride was added, and then enough aqueous sodium carbonate to precipitate all the calcium. The calcium carbonate was filtered off by a sintered glass disk. It was found that if this procedure was repeated four or five times, the rate of gas evolution fell rapidly to a constant low value; further precipitations did not reduce the rate any more. Presumably this procedure removed the copper or nickel salts by coprecipitation with the calcium carbonate. The fact that a final slow rate was reached is some evidence that this was the rate of the uncatalyzed reaction. It was checked that calcium carbonate did not affect the rate; nor did pyrex glass, since the same rate was obtained in a polyethylene flask.

Sodium Chlorite

This was the best available commercial material, manufactured by the Mathieson Alkali Works, recrystallized from water. It contained about 0.04% of chlorate, and a trace of chloride.

Sodium Carbonate

The reagent grade of sodium carbonate, manufactured by Merck Chemical Co., was recrystallized from water.

Sodium Chloride

The reagent grade of sodium chloride, manufactured by the General Chemical Co., was recrystallized from water.

Apparatus

The solutions were contained in a 1-liter flask fitted with (i) a mercury sealed stirrer, (ii) a side arm, normally closed, through which samples could be taken, and (iii) a glass capillary tube leading to a water-jacketed gas burette. The flask was immersed in a water thermostat of conventional design, which was found to maintain the temperature over long periods of time to $\pm 0.1°$ C., though the swing during any on–off cycle was only about 0.02° C. The temperature was read on a thermometer graduated to 0.1° C.

The oxygen evolved was measured in a gas burette graduated in 0.1 ml. In practice the volume could be easily read to 0.05 ml. In reducing the observed volumes to N.T.P. it was always assumed that the oxygen was saturated with water vapor at the temperature of the burette. The confining liquid was mercury, but during a run a trace of water usually condensed out of the oxygen, especially in the runs at higher temperature. The pressure of the oxygen was adjusted to atmospheric pressure, as shown by a small mineral oil manometer; the atmospheric pressure was noted to the nearest $\frac{1}{2}$ mm.

Analytical Methods

The solutions were analyzed at various times for sodium chloride, hypochlorite, chlorite, chlorate, hydroxide, and carbonate. The methods were the same as those outlined by the author in a previous paper (4).

EXPERIMENTAL PROCEDURE

The general procedure in almost all runs was as follows. A quantity of the stock hypochlorite solution was poured into the reaction flask, the amount added being weighed to the nearest 0.1 gm. Sodium chloride, sodium carbonate, or water (as required) were also weighed out and added. The final volume was always close to 900 ml. When appreciable additions were made to the stock hypochlorite solution, a sample was pipetted out and its density determined; from this the volume of solution could be found, so that the oxygen evolution per liter of solution could be calculated.

The flask was then brought to the right temperature in the thermostat, and at intervals samples were taken for analysis. Between samples the gas evolution was followed by means of the gas burette. It was found that the gas evolution was usually slower at first but built up to a practically constant rate. It is believed that this is due to the building up of a certain degree of supersaturation in the solution, and that eventually the steady rate of gas evolution equals the true reaction rate; it is always the steady rate that is reported in the following data.

The only exceptions to this procedure were in two runs intended to discover whether the pyrex flask catalyzed the reaction. In one of these the flask was filled with pyrex beads, and in the other a polyethylene flask was used. These runs are not reported in detail, but it was found that they gave the same rates as in the ordinary pyrex flask. It was also checked that calcium carbonate, which might be present in traces from the purification process, did not affect the rate. In a few runs sodium chlorite solutions were used, but the general procedure was the same.

EXPERIMENTAL RESULTS

The results for the decomposition of the stock hypochlorite solution at various temperatures are given in Table I. Table II gives various rates of gas evolution. In this table are given data on some runs which were done with filtered hypochlorite solution, but which had not had the complete calcium carbonate treatment. These gas rates are high relative to later runs, so their rate constants for oxygen evolution have little significance; however the data are needed for calculations on the rates of reaction leading to chlorate. It is believed that the rate constants of the reaction to chlorate obtained from these runs are reliable, since (i) it was found that the purification process did not affect the rate of chlorate production, but only that of oxygen, and (ii) it was found (to anticipate results which will be reported later) that catalysts such as copper oxide did not affect the rate of chlorate formation. These runs are also of interest because they show that the rate of gas formation is proportional to the hypochlorite concentration, at least in these solutions; and they throw some light on the activation energy of the (presumably) uncatalyzed reaction to oxygen. Table III shows the effect of ionic strength on the rate constant to chlorate, and Table IV shows the effect of ionic strength on oxygen evolution. Table V gives results on the effect of sodium carbonate, and sodium hydroxide. Table VI gives data on the chlorite–hypochlorite reaction.

TABLE I

Run	Temp., °C.	Time, min.	(ClO^-), *M*	(ClO_2^-), *M*	(ClO_3^-), *M*	(NaCl), *M*	Remarks
1	40	0	1.556	0.007	0.044	1.80	Ionic strength
		138	1.548	0.008	—		3.79
		289	1.545	0.0075	0.047		(NaOH) = 0.32 *M*
		581	1.532	0.008	0.051		(Na_2CO_3) = 0.02 *M*
		1244	1.506	0.0075	0.059	1.83	
2	50	0	1.497	0.0095	0.061	1.84	As in run 1
		171	1.476	0.010	0.067		
		279	1.457	0.013	0.070		
		521	1.433	0.012	0.079	1.89	
3	60	0	1.356	0.011	0.105	1.94	As in run 1
		112	1.316	0.015	0.112		
		250	1.278	0.010	0.128		
		380	1.237	0.011	0.138	2.02	
4	40	0	1.201	0.006	0.152	2.05	As in run 1
		193	1.192	0.008	0.154		
		409	1.187	0.009	0.154		
		1386	1.164	0.007	0.162	2.08	
5	60	0	1.134	0.007	0.174	2.09	As in run 1
		353	1.045	0.009	0.199		
		660	0.991	0.0075	0.219		
		1318	0.883	0.006	0.250		
		1754	0.822	0.006	0.268	2.31	
6	50	0	1.481	0.009	0.178	1.84	As in run 1
		347	1.434	0.007	0.088		
		658	1.397	0.010	0.103	1.90	
7	50	0	1.320	—	—	1.96	As in run 1
		356½	1.283				
		659	1.253				
		1441	1.185				
		2124	1.129				
		3061	1.055				
		4434	0.973				
		4975	0.943				
		5874	0.898				
		6370	0.869	—	—	2.28	
8	60	0	0.973	0.007	0.015	2.48	Ionic strength
		82	0.961				3.63
		210	0.942				(NaOH) = 0.042 *M*
		302	0.929				(Na_2CO_3) = 0.036 *M*
		443½	0.908				
		590	0.889				
		1252	0.808				
		1450	0.785	—	—	2.61	

TABLE II

Run	Temp., °C.	Mean (ClO^-), gm-mol./l.	Gas rate, ml. N.T.P./min. per l. of soln.	K_0, $min.^{-1}$
1	40	1.545	0.0405	2.34×10^{-6}
		1.525	0.0375	2.20
		1.505	0.039	2.31
4	40	1.19	0.0325	2.44
		1.16	0.0315	2.42
2	50	1.48	0.114	6.88
		1.43	0.108	6.74
7	50	1.17	0.091	6.94
		0.885	0.067	6.99
3	60	1.325	0.288	19.25
		1.295	0.279	19.2
		1.26	0.282	19.95
5	60	1.035	0.225	19.4
		0.855	0.187	19.5

TABLE III

Run	Temp., °C.	Ionic strength	Time, min.	(ClO^-), *M*	(ClO_2^-), *M*	(ClO_3^-), *M*	(NaCl), *M*	Remarks
9	60	5.83	0	0.987	0.010	0.192	4.20	(NaOH) = 0.32 *M*
			143	0.947	0.008			($NaCO_3$) = 0.02 *M*
			223½	0.927				
			427½	0.882				
			506½	0.861	0.004	0.235	4.37	
10	60	4.84	0	1.182	0.014	0.145	3.14	As in run 9
			124½	1.135				
			297	1.083				
			411	1.046	0.015	0.185	3.23	
14	60	4.71	0	1.137	0.009	0.144	3.07	(NaOH) = 0.28 *M*
			410	1.021				(Na_2CO_3) = 0.02 *M*
			1367	0.816	0.010	0.243	3.28	
19	60	4.16	0	1.719	0.022	0.029	1.95	(NaOH) = 0.29 *M*
			62	1.684	0.020	0.039		(Na_2CO_3) = 0.05 *M*
			126	1.650	0.016	0.053		
			383	1.506	0.017	0.094	2.08	
11	60	3.79	0	1.164	0.010	0.165	2.07	(NaOH) = 0.32 *M*
			394	1.077	0.010	0.191		(Na_2CO_3) = 0.02 *M*
15	60	3.69	0	0.862	0.010	0.240	2.23	(NaOH) = 0.29 *M*
			458	0.805				(Na_2CO_3) = 0.02 *M*
16	60	2.76	0	0.865	0.007	0.101	1.50	(NaOH) = 0.18 *M*
			346	0.834				(Na_2CO_3) = 0.03 *M*
			949	0.784				
			1332	0.759	0.007	0.134	1.57	
12	60	2.73	0	0.864	0.007	0.111	1.48	(NaOH) = 0.23 *M*
			144	0.854				(Na_2CO_3) = 0.01 *M*
			285	0.843				
			493	0.821				
			627	0.809	0.006	0.129	1.50	
20	60	2.61	0	0.923	0.014	0.038	1.28	(NaOH) = 0.28 *M*
			146	0.915				(Na_2CO_3) = 0.03 *M*
			349	0.895				
			1139	0.812				
			1749	0.757				
			2561	0.695	0.008	0.107	1.43	
17	60	1.74	0	0.539	0.004	0.064	0.88	(NaOH) = 0.19 *M*
			778	0.519				(Na_2CO_3) = 0.02 *M*
			2321	0.483	0.006	0.079	0.92	
21	60	1.75	0	0.710	0.006	0.028	0.88	(NaOH) = 0.06 *M*
			389	0.695				(Na_2CO_3) = 0.02 *M*
			1246	0.658				
			1828	0.638				
18	60	0.59	0	0.1625	0.002	0.0185	0.26	(NaOH) = 0.11 *M*
			733	0.161				(Na_2CO_3) = 0.01 *M*
			2296	0.158	0.003	0.019		
13	60	0.515	0	0.1630	0.002	0.023	0.27	(NaOH) = 0.05 *M*
			235	0.1616				(Na_2CO_3) = 0.00 *M*
			700	0.1553				
			1295	0.1518				
			2012	0.1494	0.001	0.026	0.29	
24	50	2.61	0	1.008	0.007	0.022	1.22	(NaOH) = 0.28 *M*
			269	0.997				(Na_2CO_3) = 0.03 *M*
			464	0.990				
			1202	0.960	0.008	0.033	1.25	

TABLE IV

DATA ON RATES OF EVOLUTION OF OXYGEN

Run	Temp., °C.	Ionic strength	Mean (ClO^-), gm-mol./l.	Gas rate, ml. N.T.P./min. per l. of soln.
9	60	5.83	0.965	0.276
			0.935	0.243
			0.87	0.241
10	60	4.84	1.16	0.247
			1.065	0.231
14	60	4.71	1.105	0.216
			1.01	0.199
15	60	3.69	0.845	0.0885
16	60	2.76	0.84	0.052
			0.77	0.050
17	60	1.74	0.52	0.028
			0.48	0.024
18	60	0.59	0.16	0.009
21	60	1.75	0.70	0.033
			0.65	0.030
22	70	1.75	0.60	0.062
23	75	1.75	0.56	0.099

TABLE V

EFFECT OF SODIUM HYDROXIDE AND CARBONATE

Run	Temp., °C.	Time, min.	(ClO^-), *M*	(ClO_2^-), *M*	(ClO_3^-), *M*	Remarks
25	50	0	1.127	0.009	0.070	Ionic strength 4.57
		307	1.102			(NaOH) = 0.28 *M*
		987	1.047			(Na_2CO_3) = 0.51 *M*
		1217	1.032	0.009	0.099	
26	50	0	1.377	0.012	0.086	Ionic strength 4.25
		380	1.337			(NaOH) = 0.07 *M*
		1356	1.233	0.011	0.133	(Na_2CO_3) = 0.265 *M*
27	60	0	1.262	0.011	0.118	Ionic strength 3.70
		186½	1.218			(NaOH) = 0.023 *M*
		292	1.189			(Na_2CO_3) = 0.019 *M*
		381	1.166	0.010	0.150	
28	50	0	1.135	0.009	0.161	Ionic strength 3.73
		222	1.119			(NaOH) = 0.275 *M*
		545	1.090			(Na_2CO_3) = 0.04 *M*
		1319	1.041			
		1686	1.014	0.009	0.192	
22	70	0	0.620	0.0065		Ionic strength 1.75
		269	0.5925			(NaOH) = 0.06 *M*
						(Na_2CO_3) = 0.02 *M*
23	75	0	0.5665			As in run 22
		147	0.550			

TABLE VI

DATA ON CHLORITE–HYPOCHLORITE REACTION
Ionic strength 3.79

Run	Temp., °C.	Time, min.	(ClO^-), *M*	(ClO_2^-), *M*	(ClO_3^-), *M*
29	40	0	0	0.762	0.002
		1784	0	0.762	0.003
30	60	0	0	0.754	0.003
		1287	0	0.755	0.003
31	50	0	1.301	0.180	0.122
		52	1.275	0.148	
		89	1.248	0.133	
		135	1.230	0.111	
		226	1.201	0.081	0.220
32	50	0	0.972	0.345	0.267
		37	0.937	0.311	
		84	0.899	0.278	
		128	0.869	0.249	
		171	0.844	0.225	
		302	0.781	0.168	
		398	0.751	0.139	
33	45	0	1.239	0.284	0.115
		65	1.202	0.248	
		148	1.165	0.210	
		224	1.133	0.184	
		318	1.096	0.146	
34	40	0	1.230	0.324	0.105
		49	1.213	0.306	
		83	1.195	0.295	
		151	1.175	0.273	
		203	1.160	0.257	
		270	1.138	0.242	
		335	1.119	0.226	
		567	1.072	0.176	
		669	1.052	0.161	

DISCUSSION OF RESULTS

It is apparent from these results that the main reaction in the decomposition of sodium hypochlorite is the one to chlorate, and only a small part goes to oxygen. Consequently the over-all kinetics are those of the reaction to chlorate, and the results of Table I fit the equation of a second order reaction: this can be seen from the long run, run 7, or by comparing runs 1 and 4, or 3 and 5. The possibility of catalysis by chloride, or effects of sodium hydroxide, will be considered later when the rate constants have been evaluated. Table II shows that for these runs the reaction to oxygen is first order, though owing to the relatively small amounts of gas evolved the rates are not very accurate. Any comparisons of this sort must be made between runs at the same ionic strength; fortunately the decomposition of hypochlorite does not change the ionic strength during a run.

The mechanism of the reaction is evidently that proposed by Foerster, with

the addition of the reaction to oxygen. Let the rate constants of the various reactions be k_1, k_2, and k_0 as follows:

$$\begin{aligned} 2NaOCl &\rightarrow NaCl + NaClO_2 && k_1 \\ NaOCl + NaClO_2 &\rightarrow NaCl + NaClO_3 && k_2 \\ NaOCl &\rightarrow NaCl + \tfrac{1}{2}O_2 && k_0 \end{aligned}$$

and let the concentrations be: $(ClO^-) = x$, and $(ClO_2^-) = y$. Then the rate equations are (assuming no effect of chloride):

[i] $$dx/dt = -k_1x^2 - k_2xy - k_0x$$

[ii] $$dy/dt = \tfrac{1}{2}k_1x^2 - k_2xy.$$

These equations are difficult to solve rigorously, but various simplifying assumptions can be made appropriate to the conditions of the reaction. In particular the chlorite concentration is low and changes very little during a run; so approximately $dy/dt = 0$. Equation [i] then becomes:

$$dx/dt = -\tfrac{3}{2}k_1x^2 - k_0x$$

whose solution is most useful in the form:

[iii] $$\frac{1}{x} = \frac{1}{x_0} + \frac{b}{k_0}(e^{k_0 t} - 1)$$

where $x = x_0$ at $t = 0$; and $b = \tfrac{3}{2}k_1 + k_0/x_0$.

To evaluate k_1 it seemed simplest to plot $1/x$ against t, and to determine the slope of the best line through the points. Calling this slope s, we get:

$$s = \frac{1/x - 1/x_0}{t} = \frac{b}{k_0 t}(e^{k_0 t} - 1) = b(1 + \tfrac{1}{2}k_0 t + \tfrac{1}{6}k_0^2 t^2 + \ldots).$$

As $k_0 t$ is always small (never above 0.04), to a good approximation:

$$s = b(1 + \tfrac{1}{2}k_0 t)$$

and

[iv] $$\tfrac{3}{2}k_1 + k_0/x_0 = s(1 + \tfrac{1}{2}k_0 t)^{-1}.$$

k_0 was determined as explained in the next paragraph, and k_1 was then found from equation [iv]. It will be seen that k_1 could also be obtained from the chlorate concentrations, and this was occasionally done, but the hypochlorite analyses are probably the more accurate.

For the determination of k_0, let v be the quantity of oxygen evolved at time t, in units of gm-atoms of oxygen per liter of solution. Then:

$$dv/dt = k_0x.$$

Putting in the value of x from equation [iii], integrating, and since $v = 0$ at $t = 0$, we get:

[v] $$v = \frac{2k_0}{3k_1} \cdot \ln\left[\frac{3k_1x_0}{2k_0}(1 - e^{-k_0 t}) + 1\right].$$

This is awkward to apply, but suppose we take as an approximate value of k_0:

$$[vi] \qquad k_0(\text{approx.}) = \frac{v/t}{\frac{1}{2}(x+x_0)},$$

i.e. the average rate of gas evolution divided by the mean hypochlorite concentration during the measuring period; then from equations [iii] and [v], expanding in powers of t up to t^2:

$$k_0(\text{approx.}) = k_0[1+\tfrac{1}{4}(k_1x_0+\tfrac{2}{3}k_0)(3k_1x_0-k_0/2)t^2] .$$

The term in t^2 gives the error introduced by this approximation for k_0. Using typical figures for 60° C., this term is about $2.5\times10^{-8}t^2$. The gas collection period never ran above 400 min., when the error is about 0.4%. Hence this approximation is sufficiently accurate. The values of k_1 and k_0 given below are from equations [iv] and [vi]. The evaluation of k_2 will be discussed in a later section.

The results in Table II give values of k_0 which are appreciably higher than those obtained for the most carefully purified solutions. Nevertheless these results are of interest as showing that the gas rate is proportional to the hypochlorite concentration (at least for these solutions); and the data are needed in evaluations of k_1. The mean k_0 values for each run are:

Run	1	4	7	2	3	5	
Temp.	40	40	50	50	60	60	° C.
k_0	2.28	2.48	6.95	6.81	19.45	19.4	$\times10^{-6}$ min.$^{-1}$

The average k_0 at each temperature was used in obtaining k_1 for these runs. It is interesting to note that although a trace of impurity must be present, these runs give an apparent activation energy of about 21 kcal./gm-mol., which is considerably higher than for any of the catalyzed reactions that were examined.

The effect of ionic strength can be obtained from Tables III and IV. From the gas rates in Table IV, we get:

Run	9	10	14	15	16	21	17	18	
Ionic strength	5.83	4.84	4.71	3.69	2.76	1.75	1.74	0.59	
k_0 (60° C.)	25.1	19.2	17.6	9.4	5.7	4.2	4.7	5.0	$\times10^{-6}$ min.$^{-1}$

k_0 varies considerably with ionic strength. As will be seen in the next section, a parallel change is also found for k_1. The results in runs 21, 22, and 23 enable k_0 to be found at different temperatures but the same ionic strength. The results are:

Temp.	60	70	75	° C.
k_0	4.2	9.2	16	$\times10^{-6}$ min.$^{-1}$

These make the activation energy about 20 kcal./gm-mol. Rather surprisingly this is lower than from runs 1 to 7, but possibly the difference is due to experimental error, for the actual rates are very small. However the value seems to be about 20 to 21 kcal. It is also surprising that the activation energy seems to be lower than for the reaction to chlorate. This makes it doubtful that we have an uncatalyzed reaction; the catalyst is not pyrex glass, nor cobalt,

nickel, or copper (which give lower activation energies). However it is impossible to be sure that traces of some unknown catalyst are not present, and there must remain this element of doubt in the interpretation of the results.

The runs in Table I give the k_1 values in Table VII. It will be seen, for instance by comparison of the rate constants of runs 3, 5, and 11, that the

TABLE VII

Run	Temp., °C.	k_1, min.$^{-1}$(gm-mol./l.)$^{-1}$	(NaCl), M		Ionic strength
			Initial	Final	
1	40	1.04 $\times 10^{-5}$	1.80	1.83	3.79
4	40	1.00	2.05	2.08	3.79
2	50	3.54	1.84	1.89	3.79
6	50	3.68	1.84	1.90	3.79
7	50	3.62	1.96	2.28	3.79
3	60	11.46	1.94	2.02	3.79
5	60	11.32	2.09	2.31	3.79
8	60	10.48	2.48	2.61	3.63

purification did not affect k_1; to anticipate results reported in another paper, addition of catalysts such as copper also did not affect k_1. Table VII also gives the sodium chloride concentrations during the runs. All these runs except run 8 were at an ionic strength of 3.79; in run 8 it was 3.63. Hence it is possible to discover whether sodium chloride has a specific catalytic effect, apart from its contribution to the ionic strength. Although the range of concentrations might perhaps have been profitably extended, these runs definitely show that sodium chloride has no effect on k_1, provided the ionic strength remains unchanged. In particular, run 8 gives a value of k_1 perhaps slightly low for this ionic strength, although the sodium chloride concentration is some 25% higher than in run 3. The long runs 5 and 7 show no sign of autocatalysis, although the sodium chloride increased 10–15% during them. Further evidence in support of this contention is provided by the runs with sodium carbonate present. At an ionic strength of 3.79 the mean rate constants are:

Temp.	40	50	60	° C.
k_1	1.02	3.61	11.4	$\times 10^{-5}$ min.$^{-1}$(gm-mol./l.)$^{-1}$

These give a good linear plot of log k_1 against $1/T$, and make the activation energy 24.8 (to the nearest 0.2) kcal./gm-mol. Foerster's constants at 25° and 90° C. give a value close to 26 kcal., but his result at 50° C. does not fall on a straight line with these values in a plot of log k_1 against $1/T$.

Turning now to the effect of ionic strength on k_1, we get from Table III for k_1 at 60° C.:

Run	9	10	14	19	11	15	8	16	12
Ionic strength	5.83	4.84	4.71	4.16	3.79	3.69	3.63	2.76	2.73
k_1	17.4	16.75	15.65	12.1	11.4	11.2	10.5	7.7	7.8

Run	20	17	21	18	13	
Ionic strength	2.61	1.74	1.75	0.59	0.515	
k_1	7.6	5.5	5.4	3.85	4.3	$\times 10^{-5}$ min.$^{-1}$(gm-mol./l.)$^{-1}$

These values give somewhat the same type of trend that might be expected for the activity coefficients of sodium hypochlorite over this range of ionic strengths. The trend in k_0 is somewhat, though not very, similar.

Run 24 makes $k_1 = 1.98 \times 10^{-5}$ min.$^{-1}$(gm-mol./l.)$^{-1}$ at 50° C. and an ionic strength of 2.61. This is proportionally a somewhat larger drop than at 60° C., but not very much. Runs 25 and 26 (Table V) give the effect of adding sodium carbonate; they make k_1 at 50° C.:

Run	25	26	
Ionic strength	4.57	4.25	
k_1	4.25	3.95	$\times 10^{-5}$ min.$^{-1}$(gm-mol./l.)$^{-1}$
(NaCl) initial	1.58	1.99	M

Relative to the more carefully measured rate at an ionic strength of 3.79 these runs show an increase in k_1 which is nearly what might be expected purely from the increase in ionic strength. The increase is perhaps a little less than in the runs when sodium chloride was added: of course specific effects are bound to occur, and one would not expect two different salts to give exactly the same change in k_1 for the same change in ionic strength. This is especially so when the two salts, as here, are not of the same ionic type. Nevertheless the main changes in k_1 do seem to be attributable to the changes in ionic strength.

Runs 21, 22, and 23 enable us to evaluate k_1 at three temperatures at another ionic strength (1.75):

Run	21	22	23	
Temp.	60	70	75	° C.
k_1	5.4	16.0	24.0	$\times 10^{-5}$ min.$^{-1}$(gm-mol./l.)$^{-1}$

The figures at the higher temperatures are rather rough; they give a moderately linear plot of log k_1 against $1/T$, with a slope corresponding to an activation energy of about 24 kcal. The experimental error is too large to say whether there is any real difference between the activation energies at ionic strengths of 3.79 and 1.75.

Table V gives the data at low sodium hydroxide concentrations. The bulk of the runs in this paper were done in the presence of 0.32 M sodium hydroxide. Some of the runs at low sodium hydroxide concentrations have already been considered, and found to fall into line with the other runs (e.g. runs 8 and 21). The k_1 values for various runs which provide evidence on the effect of sodium hydroxide are as follows:

Run	(mean)	27	8	21	17	(mean)	28	
Temp.	60	60	60	60	60	50	50	° C.
Ionic strength	3.79	3.70	3.63	1.75	1.74	3.79	3.73	
(NaOH)	0.32	0.023	0.042	0.057	0.19	0.32	0.275	M
k_1	11.4	10.9	10.5	5.42	5.48	3.60	3.48	

These results show that sodium hydroxide has a negligible effect apart from its contribution to the ionic strength. At very low sodium hydroxide concentrations hydrolysis to hypochlorous acid and its decomposition become important; but the rate of this reaction has been measured (4), and it can be calculated that the sodium hydroxide would have to be close to 0.001 M before this reaction made much difference to the total rate.

Finally the results in Table VI give information on the hypochlorite–chlorite reaction. Firstly runs 29 and 30 show that the decomposition of chlorite by itself is entirely negligible. From the remaining runs k_2 was evaluated as follows. As one molecule of chlorite removes one of hypochlorite, and since this reaction is much faster than the decomposition of hypochlorite (k_1), then approximately:

$$dx/dt = dy/dt = -k_2xy$$

where, as before, x is hypochlorite and y is chlorite. The solution of this equation is:

[vii] $$\frac{\ln(x-c)x_0}{xy_0} = \frac{\ln\ yx_0}{y_0(y+c)} = -ck_2t$$

where $c = x_0-y_0$. In reactions of this sort k_2 is normally obtained from this equation, but in the present case a small correction has to be applied for the decomposition of the hypochlorite, which is slow but not negligible in comparison. It therefore seemed simpler to take an approximate value of k_2 defined by

$$k_2(\text{approx.}) = \text{corrected rate}/(\text{mean } x)(\text{mean } y)\ .$$

By "corrected rate" is meant dx/dt between any two successive readings minus the part of the rate due to decomposition of hypochlorite, which can be calculated from k_1. If the values of x and y from equation [vii] are substituted in this expression, and the exponentials expanded in powers of t, we get:

[viii] $$k_2(\text{approx.}) = k_2[1-(x_0^2+x_0y_0+y_0^2)(k_2^2t^2/12)+\ \ldots\ ]\ .$$

This gives the error in k_2 in this approximation: in the runs reported it rose to about 1%, but the error could then be allowed for. To examine the validity of our method of allowing for k_1 and k_0, we can subtract equations [i] and [ii] to get:

$$dy/dt-dx/dt = \tfrac{3}{2}k_1x^2+k_0x\ .$$

Substituting the value of x from [vii], integrating, and expanding in powers of t, it is found that the fractional error in k_2 so introduced is:

$$\text{Fractional error} = \frac{(6k_1y_0+3k_1x_0-k_0)}{3k_1x_0+2k_0}\ .\ x_0y_0k_2t.$$

The size of this error was checked for runs 31 to 34, and in no case was above 0.3%. Consequently it was assumed to be adequate to obtain k_2 simply by taking the observed slope of x (or y) over each interval between analyses, correcting for the decomposition of hypochlorite if the slope of x is used, and dividing the slope by the mean x times the mean y over the interval. A small correction was applied in accordance with equation [viii]. The mean values of k_2 for each run were:

Run	31	32	33	34	
Temp.	50	50	45	40	°C.
k_2	2.75	2.77	1.64	0.97	$\times10^{-3}$ min.$^{-1}$(gm-mol./l.)$^{-1}$

These results give an activation energy of 20.8 kcal. In all these runs the ionic strength was 3.79, and the sodium hydroxide concentration was 0.32 *M*. Foerster's results made the activation energy 20½ kcal., in good agreement. However his constant at 50° C. is about 8.3×10^{-4}, but the difference may be due to a different ionic strength. These values of k_2 mean that a decomposing sodium hypochlorite solution will contain about 1/100 as many chlorite ions as hypochlorite ions at 40° C.; this was roughly found. If the constants k_1 and k_2 are written as $Ae^{-E/RT}$, the A factors are fairly similar:

$$k_1 = 2.1\times10^{12}.e^{-24.8\ \text{kcal.}/RT}; \quad k_2 = 3.2\times10^{11}.e^{-20.8\ \text{kcal.}/RT}.$$

To summarize these conclusions, it is believed that these results show that (i) the mechanism of Foerster *et al.* via chlorite is well established; (ii) the first stage to chlorite has an activation energy of 24.8 kcal., and is slower than the second stage, which has an activation energy of 20.8 kcal.; (iii) the rates are strongly influenced by the ionic strength; (iv) added sodium hydroxide, carbonate, or chloride changes the rate by changing the ionic strength, but apart from this they exert no specific catalytic effect; and (v) there is a simultaneous unimolecular decomposition to oxygen, which is possibly, but not certainly, uncatalyzed.

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DECOMPOSITION OF SODIUM HYPOCHLORITE: THE CATALYZED REACTION[1]

By M. W. Lister

ABSTRACT

The catalyzed decomposition of sodium hypochlorite has been examined; the catalysts tried were manganese, iron, cobalt, nickel, and copper oxides. It was shown that in no case was the decomposition to chlorate and chloride accelerated, only the reaction to chloride and oxygen. Manganese and iron did not catalyze even the latter reaction, or only to a very small extent; this was in fairly concentrated sodium hypochlorite containing some sodium hydroxide. The manganese and iron are largely oxidized to permanganate and ferrate under these conditions. It was found that copper could catalyze the formation of permanganate and ferrate, and nickel the formation of permanganate. Cobalt catalyzed the reaction going to oxygen, and the rate was proportional to the cobalt added, but little dependent on the hypochlorite concentration; the same is true of nickel. Copper (as reported earlier) gives a catalyzed reaction not far from first order in hypochlorite. The activation energies were measured, and were consistent with the relative catalytic activity of these metals. The mechanism of the reaction is briefly discussed.

The decomposition of sodium hypochlorite is catalyzed by the oxides of transition elements such as cobalt. Qualitatively this was first known a long time ago, but quantitative measurements on the catalysis are much more recent. Howell (3) made a careful study of the catalysis by cobalt oxide. He added the cobalt oxide as a fine, almost colloidal, suspension in water, and measured the rate of oxygen evolution from the vigorously stirred mixture. At fairly low catalyst concentrations (about 2 mgm. cobalt in 60 ml. of solution), he found the rate to be proportional to the catalyst and to the hypochlorite concentrations. The activation energy of the catalyzed reaction was 16.6 kcal./gm-mol. Moelwyn-Hughes (10) was able to calculate that Howell's rate constants are fairly near what one would expect if the catalyst were present as particles of 5×10^{-7} cm. diam., and if every collision with a hypochlorite ion with sufficient energy were effective. Chirnoaga made somewhat similar measurements for cobalt and nickel oxides (1). He found the rate to be independent of the stirring speed, provided this was moderately rapid. His rates were not proportional to the hypochlorite concentration: with nickel oxide they were proportional to $(ClO^-)^{0.47}$ at low concentrations (0.02 to 0.04 M). With cobalt oxide the rate was proportional to $(ClO^-)^{0.9}$. The activation energy was 15.8 kcal. for cobalt oxide, and 16.6 kcal. for nickel oxide. Both Howell and Chirnoaga reported that if the catalyst was washed for a long time till all alkali was removed, it was more active. This may be a peptizing effect due to removal of electrolyte. However all the hypochlorite solutions used contained free alkali. Chirnoaga reported that the order of catalytic activity was Ni > Co > Cu > Fe > Mn, although nickel and cobalt might be expected to be in the reverse order from the activation energies.

Dixon and White (2) examined the conversion of Mn(II) to permanganate by hypochlorite, and noted that copper could catalyze this. Lewis (5, 6) examined cobalt, copper, and iron oxides as catalysts. At fairly high concen-

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Contribution from the Department of Chemistry, University of Toronto, Toronto, Ontario.

trations he found the rate to be independent of the hypochlorite concentration, and it seems difficult to reconcile his results with those of Howell. He was also particularly interested in the effect of adding substances, ordinarily inert, as promoters.

The present author (8) made measurements on the catalysis by copper, during some investigations of the complex copper tellurates and periodates. As these results were obtained under conditions comparable to the measurements reported in this paper, they will be discussed briefly later.

One of the difficulties in interpreting the results of these measurements (either the earlier or the present work) is the uncertainty in the size of the catalytic particles. The apparent gross size is, of course, no certain indication of the true surface area, as pores are probably present. Comparisons with previous rate measurements always contain this element of uncertainty. Consequently the present work was confined to some more limited matters about the reaction. Firstly, although the decomposition to sodium chloride and oxygen is certainly catalyzed, it is not clear whether the decomposition to sodium chloride and chlorate is similarly catalyzed. Secondly, it has sometimes been suggested that qualitatively the mechanism of catalysis is through unstable higher oxides, which are formed, decompose, and are reformed by further oxidation, and so on. However manganese and iron do not have such unstable higher oxides, so their degree of catalytic activity was investigated. Thirdly, the energies of activation of all the catalyzed reactions were measured again. Finally, some further evidence on the dependence on hypochlorite concentration was obtained, which supports Lewis's views at least over a certain range of conditions; but there are several factors which can affect the rate, and a complete study of the matter would require a great many experiments.

EXPERIMENTAL METHOD

The materials used were the same as in the previous paper, with the addition of the salts of various metals used as catalysts. Reagent grade samples of manganese chloride, ferric nitrate, cobalt chloride, nickel chloride, and copper chloride were used. Stock solutions of these salts were made up, and various amounts were pipetted out, and added to well-stirred alkaline sodium hypochlorite. Precipitates of the oxides immediately formed, except with copper. These precipitates were finely divided, but not colloidal; as far as possible the conditions of addition were the same each time. The solutions were then brought to the desired temperature in a thermostat, with continual stirring, and the rate of gas evolution was measured. Samples were taken for analysis from time to time. The apparatus was the same as described in the first paper on the uncatalyzed reaction (9). The analytical methods have been described in another previous paper (7).

The sodium hypochlorite was the same as in the investigation of the uncatalyzed reaction; its ionic strength was 3.79, and it was 0.32 *M* in sodium hydroxide.

EXPERIMENTAL RESULTS AND CONCLUSIONS

Before describing the quantitative runs, a few qualitative observations might be included. Dixon and White (2) observed that copper catalyzed the

formation of permanganate. Manganese dioxide is, of course, first formed, starting with Mn(II); and even without copper, permanganate is formed slowly at room temperature. It was noticed that nickel also catalyzed the formation of permanganate, though less efficiently than copper. Sodium chlorite does not oxidize Mn(II) beyond manganese dioxide.

Iron is similarly slowly oxidized to ferrate in the cold, and quite rapidly at 80° C.; as would be expected the reaction goes faster in strongly alkaline solution. Although the ferrate is unstable on boiling, it decomposes only very slowly at 60° C. Copper also accelerates the formation of ferrate, but nickel seems to be unable to do this. The qualitative behavior of copper was described in the paper mentioned earlier (8). As is well known, cobalt and nickel are oxidized to higher oxides by sodium hypochlorite, which are almost completely insoluble in the alkaline solution. Sodium chlorite will not accomplish any of these oxidations.

In the quantitative runs, it is probably simplest to consider each element separately; the results will be given and any conclusions drawn from them before going on to the next element.

Manganese

The data on manganese as a catalyst are given in Table I. The conclusion that must be drawn from these results is that manganese does not catalyze

TABLE I

Run	Temp., °C.	Mn added, mgm.	Solution volume, ml.	Time, min.	(ClO^-), *M*	(ClO_2^-), *M*	(ClO_3^-), *M*
1	50	3.32	796	0	1.285	0.009	0.122
				286	1.261	0.008	0.128
2	60	3.32	786	0	1.240	—	—
				278	1.171		

Run	Temp., °C.	Mean (ClO^-), *M*	Gas rate, ml. N.T.P./min. per liter soln.
1	50	1.273	0.0568
2	60	1.21	0.145

the reaction at all. If we use the same equation that was applied to the uncatalyzed runs (9),

$$-dx/dt = \tfrac{3}{2}k_1x^2 + k_0x,$$

where $x = (ClO^-)$,

k_1 = rate constant for first stage of reaction to chlorate,

k_0 = rate constant for reaction to oxygen,

then the results above give for the constants:

Temp.	50	60 °C.
k_2	4.0	10.7×10^{-6} min.$^{-1}$
k_1	3.25	10.75×10^{-5} (gm-mol./l.)$^{-1}$ min.$^{-1}$

By "reaction to chlorate" is meant the reaction

$$3OCl^- \rightarrow ClO_3^- + 2Cl^-$$

of which the first step is

$$2OCl^- \rightarrow ClO_2^- + Cl^-.$$

This is the slow rate determining step, and k_1 is its rate constant. By "reaction to oxygen" is meant the reaction

$$2OCl^- \rightarrow 2Cl^- + O_2.$$

This was found to be a first order reaction, and k_0 is its rate constant. These reactions are the ones meant in all references in later sections to "reaction to chlorate" or "reaction to oxygen".

The ionic strength of the solution after addition of catalyst was 3.78. The k_0 values are just about right for this ionic strength, and make the energy of activation 21 kcal., in agreement with results obtained earlier. The k_1 values are a little low, but certainly show that manganese does not catalyze the reaction under these conditions. The reason for the difference between these results and those of Chirnoaga probably lies in the higher concentration and sodium hydroxide content of the present solutions. Chirnoaga's sodium hypochlorite was only 0.042 *M*, and his manganese dioxide was 0.0061 *M*, so an appreciable fraction of the hypochlorite could be used up in oxidizing the manganese; indeed over half of the decomposition that he observed could be explained in this way. However the present results do show that manganese is not catalytic in concentrated solutions of hypochlorite.

Iron

The data on iron as a catalyst are given in Table II. Again we can treat these results by the method for uncatalyzed runs, which gives values for the rate constants as shown below. Values of k_1 from the chlorate analyses are also included, as these help to distinguish the catalytic effect (if any) on the two reactions. It may be added that constants obtained from the chlorate analyses have the advantage of not needing a value of the gas rate for their

TABLE II

Run	Temp., °C.	Fe added, mgm.	Time, min.	(ClO^-), *M*	(ClO_3^-), *M*	(ClO_2^-), *M*
3	50	3.84	0	1.256	0.008	0.131
			439	1.216	0.008	—
			1157	1.158	0.008	0 162
			1391	1.138	0.008	—
4	60	3.84	0	1.126	0.010	0.167
			462	1.030	—	—
			1377	0.873	0.009	0.246

Run	Temp., °C.	Mean (ClO^-), *M*	Gas rate, ml. N.T.P./min. per liter soln.
3	50	1.19	0.070
4	60	1.11	0.159
		0.90	0.114

calculation; but against this must be set the fact that the chlorate concentrations are obtained by the difference of two analyses, one of which does not have a very easily observed end point. The values of the constants are:

Temp.	50	60 ° C.
k_0	5.2	12.0×10^{-6} min.$^{-1}$
k_2	3.59	11.6×10^{-5} (gm-mol./l)$^{-1}$ min.$^{-1}$
k_1 (from chlorate)	3.70	11.5×10^{-5} (gm-mol./l.)$^{-1}$ min.$^{-1}$

There may be some catalytic effect on the reaction to oxygen, but it is very slight. As compared with, for instance, cobalt, the iron is at most 1/200 times as active as a catalyst. There is no sign of any catalysis of the chlorate reaction: the uncatalyzed rate constants at this ionic strength (3.79) are 3.61×10^{-5} at 50° C., and 11.4×10^{-5} at 60° C. We must add, of course, that these conclusions only apply to the conditions of these experiments; that is to say in fairly high hypochlorite concentrations, moderate alkalinity, and not too high temperatures; but under these conditions iron does not catalyze the chlorate reaction, and it catalyzes the oxygen reaction very slightly, if at all.

Cobalt

The data obtained are given in Table III. Cobalt, of course, catalyzes the oxygen reaction, but its effect on the chlorate reaction is not so obvious. The rigorous determination of k_1 from these data is not easy because of the high

TABLE III

Run	Temp., ° C.	Co added, mgm.	Time, min.	(ClO^-), *M*	(ClO_2^-), *M*	(ClO_3^-), *M*
5	50	3.16	0	1.246	0.001	0.134
			488	1.074	—	0.146
			1262	0.854	0.003	0.160
6	50	1.58	0	1.229	0.002	0.139
			440	1.109	0.004	0.150
			1338	0.924	0.002	0.167

Run	Temp., ° C.	Mean (ClO^-), *M*	Gas rate, ml. N.T.P./min.	Co added, mgm.
5	50	1.235	2.47	3.16
		1.065	2.35	
6	50	1.17	1.24	1.58
		1.08	1.02	
7	50	0.91	0.87	1.58
		0.50	0.81	
8*a*	30	1.25	0.51	3.16
8*b*	40	1.23	1.08	3.16
8*c*	50	1.18	2.46	3.16
8*d*	45	1.03	1.87	3.16
9*a*	30	1.25	0.225	3.16
9*b*	40	1.23	0.59	3.16
9*c*	50	1.19	2.48	3.16
9*d*	45	1.05	1.84	3.16
9*e*	40	0.98	1.16	3.16
9*f*	35	0.94	0.71	3.16
9*g*	30	0.91	0.49	3.16

gas rate. However a reasonable approximation can be obtained by observing the total rate of decomposition between any two analyses; we then put:

$$\text{total rate} = \text{rate to oxygen} + \text{rate to chlorate}$$

and:

$$\text{rate to chlorate} = \tfrac{3}{2}k_1\bar{x}^2$$

where $\bar{x}$ is now the mean hypochlorite concentration between the two analyses. Hence k_1 can be calculated. The error from taking this approximate equation, and not an integrated equation, is small. This was checked by integrating the above equation for various plausible expressions for the dependence of the rate to oxygen on x; then the approximate k_1 could be expressed in terms of the true k_1 and known quantities such as x or the time. This enables an estimate of the error in the approximate k_1 to be made, and in the actual runs this never exceeded 1%. Although k_1 could have been obtained from these integrated equations, they were rather cumbersome expressions; and it was felt that the approximate equation allowed for the actual observed gas rate in a more direct manner.

Considering first runs 5 and 6, we get the following values of k_1, averaged over the entire run (ionic strength 3.79):

Run	k_1 from ClO^-		k_1 from ClO_3^-	
5	3.74	$\times 10^{-5}$	3.68	$\times 10^{-5}$ (gm-mol./l.)$^{-1}$ min.$^{-1}$
6	3.66		3.61	

These values are near enough to the uncatalyzed rate constant (3.61×10^{-5}) to be within the experimental error. Hence cobalt does not catalyze the chlorate reaction to a detectable extent.

If we look at the oxygen reaction, a comparison of runs 5 and 6 shows that at the same hypochlorite concentration, the rate is proportional to the cobalt added. This agrees with all previous work. The dependence on hypochlorite is more obscure, but a few tentative remarks can be made. Firstly, it is difficult to distinguish, during any one run, an 'aging' effect in the catalyst from a dependence of the rate on the hypochlorite concentration. Howell avoided this difficulty in some of his runs by filtering off a catalyst at the end of a run and re-using it; he found its activity had fallen a few per cent. The 'aging' is presumably due to agglomeration of the catalyst particles, thereby reducing their surface area; or to poisoning of the catalyst by impurities. Howell found that typical catalyst poisons had no effect, so probably changes were due to agglomeration. The following observation supports this view. It was found that if a catalyst was used for some time, then left overnight without stirring, and stirring was started next day at a fairly low temperature, then the rate of gas evolution was low (run 9*a* at 30° C.). If the temperature was increased in steps, then the rate rose towards what was found for fresh catalyst, and at 50° C. the catalyst had recovered (runs 9*b*, 9*c*). The simplest explanation is that the vigorous gas evolution at 50° C. had broken up the agglomerated particles. If the temperature was subsequently decreased, the fresh catalyst rate was more or less maintained (compare runs 8*a*, 9*g*), at least for long enough

to observe the gas rate. Hence it is impossible to say with any confidence whether the fall in rate during runs 5 and 6 is due to aging of catalyst or fall in (ClO^-). However run 7 does provide some evidence on this, as the two rates in this run were measured on the same solution to which water was added between the measurements. The rate only fell 7% for a decrease in hypochlorite concentration of 45%. Similarly a comparison of runs 5, 8*c*, and 9*c* shows nearly the same rates over a moderate range of concentration. On the whole it seems fair to conclude that, under the conditions of these experiments, the rate is very little dependent on hypochlorite, in agreement with Lewis.

This in no sense contradicts Howell's results for his experimental conditions. The difference does not lie apparently in the concentrations used, which were much the same as in the present work. The difference is probably in the method of preparation of his catalyst: per milligram his catalyst was about three times as active as that in Lewis's or the present work. Even so the degree of difference seems surprising, and the matter cannot be regarded as settled.

Runs 8 and 9 give the temperature dependence of the rate; they make the energy of activation 15.9 (±0.5) kcal./gm-mol. This is to be compared with 15.8 kcal. found by Chirnoaga, 16.6 kcal. by Howell, and (in effect) 13.3 kcal. by Lewis.

TABLE IV

Run	Temp., °C.	Ni added, mgm.	Time, min.	(ClO^-), *M*	(ClO_2^-), *M*	(ClO_3^-), *M*
10	40	3.39	0	1.369	0.006	0.095
			164	1.352	0.004	0.104
			409	1.329	0.004	0.106
			1228	1.266	0.004	0.112
11	50	3.39	0	1.243	0.003	0.115
			366	1.173	0.004	0.125
			1322	1.000	0.005	0.145
12	60	3.39	0	0.960	0.005	0.148
			320	0.833	0.003	0.164
13	50	0.85	0	1.315	0.008	0.116
			230	1.280	0.008	0.123

Run	Temp., °C.	Mean (ClO^-), *M*	Gas rate, ml. N.T.P./min.	Ni added, mgm.
10	40	1.36	0.47	3.39
		1.33	0.52	
		1.27	0.50	
11	50	1.23	1.23	3.39
		1.16	1.11	
		1.02	1.17	
12	60	0.95	2.63	3.39
		0.84	2.65	
13	50	1.30	0.321	0.85
		1.28	0.333	
14	50	0.82	1.17	3.39
		0.81	1.16	

Nickel

The data on nickel are given in Table IV. The first point to settle is whether nickel catalyzes the chlorate reaction. If we calculate the rate constant, k_1, as for cobalt, the result is:

Run	Temp., ° C.	k_1 from ClO^-	k_1 from ClO_3^-
10	40	1.35 $\times 10^{-5}$	1.29 $\times 10^{-5}$ (gm-mol./l.)$^{-1}$ min.$^{-1}$
11	50	3.52	3.62
12	60	10.5	13.0

The experimental error at 40° C. is fairly large, as an error of 0.001 in the hypochlorite concentration would alter k_1 by nearly 2½%. However it seems possible to conclude that within the experimental error, nickel does not catalyze the chlorate reaction. Runs 11 and 13 show that the rate is proportional to the nickel added, in agreement with all previous work.

The dependence of the catalyzed rate on hypochlorite concentration is best seen from runs 11, 12, and 14. Over the range of concentrations examined (0.81 to 1.23 *M*) there was no observable change in the catalyzed rate with hypochlorite concentration. Again this does not mean that under other conditions such a dependence might not be found. Chirnoaga found the rate to be proportional to $(ClO^-)^{0.47}$, at low concentrations; this is a lesser degree of dependence on concentration than he found with cobalt.

The mean gas rates at about 1 *M* hypochlorite, with 3.39 mgm. of nickel, at various temperatures are:

Temp.	40	50	60	° C.
Gas rate	0.50	1.17	2.64	ml./min.

These make the activation energy 17.2 kcal./gm-mol. A little (about 4%) of the total rate can be attributed to the uncatalyzed reaction, and if allowance is made for this, the activation energy is reduced to 17.1 kcal.

Copper

In another paper (8), the writer has given details of the effect of copper as a catalyst. The only point that will be dealt with here is whether copper catalyzes the reaction to chlorate. Table V gives the experimental results. From these results we can calculate k_1, using interpolated values of the rate of oxygen evolution at the appropriate hypochlorite concentration. The values so obtained are:

Run	15	16	17(0–1162′)	17(1162–2921′)	18	19	
k_1 from (ClO^-)	3.88	3.61	3.35	3.65	3.82	3.44	$\times 10^{-5}$
k_1 from (ClO_3^-)	4.08	—	3.82	3.60	—	—	$\times 10^{-5}$

The long run (17) was arbitrarily divided into two parts for this calculation as the range of concentration is large, but similar results would be obtained from other time intervals in this run. The accuracy of k_1, as obtained above, is not very great, as small changes in the analytical results would give relatively large changes in k_1. However it seems reasonably certain from these results that copper does not catalyze the reaction to chlorate.

As mentioned above, the dependence of rate on concentration has been reported by the writer in an earlier paper on another aspect of the chemistry

TABLE V

Run	Cu added, mgm.	Time, min.	(ClO^-), *M*	(ClO_3^-), *M*
15	3.92	0	1.250	0.120
		196	1.094	0.1255
16	3.92	0	1.018	—
		57½	0.979	—
17	1.96	0	1.201	0.124
		426	1.058	0.141
		1162	0.8325	0.157
		2595	0.500	—
		2921	0.4355	0.170
18	1.96	0	1.240	—
		543	0.940	—
19	1.96	0	0.982	—
		493	0.839	

Run	Mean (ClO^-), *M*	Gas rate, ml. N.T.P./min.
15	1.23	6.39
16	1.01	5.67
	0.99	5.33
17	1.05	2.42
	0.81	2.12
	0.71	1.97
	0.44	1.45
18	1.09	2.75
19	0.95	2.25
	0.84	2.00

of copper. Briefly, it was found that the rate could best be represented by an equation of the form:

$$\text{rate} = (\text{Cu})\{a+b(ClO^-)\}$$

where a and b are constants, and b is larger than a. The over-all energy of activation was 15.5 kcal./gm-mol.

GENERAL REMARKS

Comparison of the results with various metals enables us to make a few general comments on these reactions. In the first place, it was found that in no case was the chlorate reaction catalyzed (or only to a very small extent). This suggests that the mechanism of the catalysis is through oxidation of the metal to a higher oxide, which then loses oxygen, and is subsequently re-oxidized, and so on. The inertness of iron and manganese where there is no unstable higher oxide supports this view. Unfortunately, if we calculate the rate of decomposition of the higher oxide which would provide the observed gas evolution, it seems to be impossibly high. For instance in run 5, 5.4×10^{-5} gm-atoms of cobalt gave 2.2×10^{-4} gm-atoms of oxygen per minute. If the

change is from Co(III) to Co(II), this means that the half life of the oxidized cobalt atom is only 5.2 sec., even if all the Co(II) is instantaneously re-oxidized. As $Co_2O_3.H_2O$(or CoO.OH) is reported (4) to have been isolated, and to be stable on drying at 150° C., the above theory of the reaction seems to be untenable. Similar remarks apply, though with somewhat less force, to nickel and even to copper. Of course, we cannot entirely dismiss the possibility that the reaction is due to some still higher oxide, but this is hypothetical.

The only alternative seems to be that the reaction goes in the stages (M is any catalytic metal):

$$\text{(i)}\quad 2MO + ClO^- \rightarrow M_2O_3 + Cl^-$$
$$\text{(ii)}\quad M_2O_3 + ClO^- \rightarrow M_2O_3.ClO^- \text{ (adsorbed)}$$
$$\text{(iii)}\quad M_2O_3.ClO^- \rightarrow 2MO + Cl^- + O_2$$

This, of course, was realized by earlier workers on the reaction. If the reaction rate is independent of the hypochlorite concentration, then (iii) must be the slow stage, and much the same amount of hypochlorite must be adsorbed in (ii) over a range of concentration. While there is really no direct evidence of this, the simplest picture is one in which the entire catalyst surface is kept covered with adsorbed hypochlorite ions at the rather high concentrations actually used. If the catalyst surface is not completely covered, it is impossible to predict what the reaction kinetics would be without knowing the dependence of adsorption on concentration. Only if the adsorption were proportional to the hypochlorite concentration could we expect a first order reaction; this might happen in dilute solutions. Thus cobalt and nickel, at the concentrations used, behave as though the catalyst were fairly completely covered with adsorbed ions. Copper is entirely, or nearly entirely, in solution; and the reaction, as might be expected, is not far from being first order in both copper and hypochlorite. Manganese and iron do not catalyze the reaction, and this is at least in part attributable to their oxidation to stable states.

It is interesting to compare the rates with the activation energies. At 50° C., 10^{-4} gm-atoms of metal in 1 *M* sodium hypochlorite would give gas rates as follows:

Metal	Co	Ni	Cu	
Rate	4.0	2.0	7.4	ml./min.
Activation energy	15.9	17.1	15.5	kcal./gm-mol.

Thus the rate increases as the activation energy decreases. The rate with nickel seems somewhat large compared with the others, as the activation energy is over 1 kcal. larger.

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THE REACTION BETWEEN CYANATE AND HYPOCHLORITE[1]

By M. W. Lister

ABSTRACT

The reaction between sodium hypochlorite and potassium cyanate in the presence of sodium hydroxide has been examined. The main products are chloride, and carbonate ions and nitrogen; but, especially if much hypochlorite is present, some nitrate is formed as well. The rate of reaction is proportional to the cyanate and hypochlorite concentrations, but inversely proportional to the hydroxide concentration: the rate constant is 5.45×10^{-4} min.$^{-1}$ at 65° C., at an ionic strength of 2.2. The rate constant increases somewhat as the ionic strength rises from 1.7 to 3.5. The effect of temperature makes the apparent activation energy 25 kcal./gm-molecule. The kinetics of the reaction suggest that the slow step is really a reaction of hypochlorous acid and cyanate ions, and possible intermediate products of this reaction are suggested. Allowing for the different extent of hydrolysis of hypochlorite at different temperatures, the true activation energy is found to be 15 kcal./gm-mol., which is consistent with the observed rate of reaction.

In a recent paper (4), the writer reported some observations on the oxidation of potassium cyanate by sodium hypochlorite in the presence of small amounts of alkali. It was found that there was at first a marked induction period, after which a rapid reaction took place which approximately followed the equation:

$$2OCN^- + 3OCl^- + H_2O \rightarrow 2HCO_3^- + 3Cl^- + N_2.$$

The time required for the rapid reaction to develop depended on the concentration of sodium hydroxide, and was much less at low hydroxide concentrations. It was suggested that the acceleration of the reaction was due to the liberation of large amounts of hypochlorous acid, which oxidized the cyanate rapidly. It has since been found that the pH when the reaction accelerates is about 10. This is much too high a pH for cyanic acid to be formed, but would be reasonable for the liberation of hypochlorous acid. The reaction produces bicarbonate ions which are acid enough to lower the pH below this critical value.

A slow reaction took place even in the presence of excess alkali, and it is this reaction that is the subject of the present paper. The products are carbonate and chloride ions and nitrogen, at least in the predominant reaction. It is hardly likely that a complicated reaction of this sort would go in one step; and it was hoped that a knowledge of the kinetics would help to elucidate the mechanism, for there are no very obvious intermediate products. It is thought that the present work provides a fair amount of information on the reaction, though a detailed mechanism cannot yet be given.

EXPERIMENTAL METHOD AND RESULTS

The reagents used in these experiments were as follows. The same potassium cyanate was used as in the preceding paper (4). Sodium hypochlorite solution

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Contribution from the Department of Chemistry, University of Toronto, Toronto, Ontario.

was made in the usual way by passing chlorine into cold aqueous sodium hydroxide. A reagent grade of potassium hydroxide was used to control the hydroxide concentration. In some runs reagent grade sodium chloride or carbonate was added to alter the ionic strength.

The general procedure in any run was as follows. Solutions of potassium cyanate with added potassium hydroxide were made up as required. To these were added varying amounts of sodium hypochlorite solution, and in most runs solid sodium chloride or carbonate also, to control the ionic strength. The reaction mixture was contained in a 1 liter flask, fitted with (i) a mercury sealed stirrer, (ii) a side arm, normally closed, for taking samples, and (iii) a capillary lead to a water jacketed gas burette. The flask was immersed in a water thermostat of conventional design, whose temperature was controlled to $\pm 0.1°$ C.

During a run samples were taken at intervals and analyzed. Three sorts of analysis were used to follow the reaction: these were for (i) hypochlorite, (ii) cyanate, and (iii) total alkalinity. In addition the stock hypochlorite solution was analyzed for chlorite, chlorate, hydroxide, and carbonate, as it was necessary to know its ionic strength. The methods, except for cyanate and total alkalinity, have been described in an earlier paper (5). These are all well known titrations with minor modifications. To determine cyanate, a sample was run into 20 ml. of 3% hydrogen peroxide, to destroy hypochlorite. The mixture was acidified, and heated nearly to boiling to convert all cyanate to ammonium ions. Excess alkali was then added, and the ammonia distilled into a known amount of standard sulphuric acid, and the remaining acid was titrated in the usual way. 'Total alkalinity' was determined by the following procedure. A sample was pipetted out and titrated with standard sulphuric acid. Near the end point the solution was heated to boiling, and acid was run in until the green color of bromcresol green, which was used as indicator, was maintained even in the hot solution. At this point all hydroxide and carbonate have been titrated (the latter to carbonic acid), and the cyanate just converted to ammonium ions and carbon dioxide:

$$2KOCN + 2H_2SO_4 + 2H_2O \rightarrow K_2SO_4 + (NH_4)_2SO_4 + 2CO_2.$$

In one run the reaction of sodium chlorite and potassium cyanate was examined. This was done because hypochlorite always contains a small amount of chlorite, and it seemed possible that a reaction of this sort might contribute appreciably to the total rate. The procedure was the same except that a solution of recrystallized sodium chlorite was used.

The results are given in Tables I to V, which are arranged as follows. Table I gives the main series of runs, which were made to determine the kinetics of the reaction. All these runs were at 65° C., and an ionic strength of 2.2. Table II gives the results of varying the ionic strength, and Table III the results of varying the temperature. The observations on gas evolution are given in Table IV. Finally the single run with chlorite is given in Table V.

TABLE I

All runs at 65° C. and an ionic strength of 2.2

Run	Time, min.	(ClO^-), *M*	(OCN^-), *M*	Total alkalinity, *N*	(NaOH), *M*
1	0	0.2495	0.313	0.913	0.286
	41	0.235	0.299	—	
	111	0.2005	0.284	0.875	
	201½	0.1645	—	—	
	317	0.1285	—	0.815	
	361	0.116	0.234	—	
2	0	0.461	0.304	0.965	0.357
	65	0.419	0.285	0.929	
	114	0.387	—	0.915	
	190	0.347	0.250	0.895	
	279	0.2925	—	0.873	
	300	0.243	0.194	0.856	
3	0	0.715	0.307	1.010	0.396
	52	0.661	0.292	0.988	
	104	0.615	0.265	0.959	
	185	0.535	0.232	0.929	
	277	0.4735	0.200	0.900	
	353	0.424	0.167	0.876	
4	0	0.2555	0.628	1.536	0.280
	42	0.2115	—	1.504	
	124	0.1455	0.561	1.495	
	190	0.104	0.533	1.465	
	261	0.071	0.513	1.456	
	317	0.0505	0.505	1.442	
5	0	0.3005	1.129	2.553	0.296
	45½	0.213	1.040	2.312	
	91½	0.1485	0.984	2.273	
	176½	0.063	0.937	2.258	
	254	0.0215	0.915	2.255	
6	0	0.299	0.106	0.526	0.314
	200	0.268	0.0925	0.512	
	330	0.250	0.080	0.496	
7	0	0.2955	0.329	1.561	0.245
	66	0.257	0.305	1.546	
	150	0.2085	0.281	1.512	0.329 *M*
	272	0.1545	0.250	1.484	Na_2CO_3
	346	0.1275	0.233	1.470	
8	0	0.294	0.328	1.192	0.536
	67	0.270	0.318	1.174	
	177	0.239	0.297	1.160	
	312	0.2035	0.280	1.143	
	383	0.192	—	—	
9	0	0.293	0.321	1.796	1.13
	207	0.267	0.307	1.760	
	315	0.2485	—	—	
	374	0.2385	0.290	1.748	
10	0	0.2835	0.317	0.838	0.205
	79	0.2285	0.286	0.813	
	147	0.1905	0.262	0.795	
	229	0.1445	0.237	0.766	

TABLE II

All runs are at 65° C.

Run	Ionic strength	Time, min.	(ClO^-), *M*	(OCN^-), *M*	(NaOH), *M*
11	1.7	0	0.453	0.382	0.295
		61	0.4075	0.361	
		133	0.3625	0.327	
		185	0.3295	0.305	
		280	0.270	0.271	
		345	0.2405	0.242	
12	2.0	0	0.450	0.166	0.271
		77	0.416	0.159	
		183	0.3755	0.138	
		298	0.340	0.115	
		368	0.327	0.100	
13	2.9	0	1.037	0.324	0.503
		53½	0.977	0.303	
		117	0.900	0.272	
		205	0.803	0.235	
		289	0.731	0.198	
14	3.5	0	0.211	0.355	0.349
		100	0.1715	0.332	
		227	0.1305	0.308	
		299	0.115	0.301	

TABLE III

All runs at an ionic strength of 2.2

Run	Temp., ° C.	Time, min.	(ClO^-), *M*	(OCN^-), *M*	(NaOH), *M*
15	55	0	0.294	0.322	0.283
		124	0.265	0.304	
		265	0.2385	0.293	
		403	0.2165	0.286	
16	75	0	0.252	0.298	0.325
		49	0.193	0.277	
		112	0.135	0.245	
		174	0.0945	0.229	
		256	0.059	0.2035	
17	50	0	0.468	0.287	0.386
		60	0.459	—	
		125	0.453	—	
		450	0.425	—	
		1129	0.360	—	
		1606	0.3145	—	
		1620	0.3125	—	
		2548	0.257	—	
		3016	0.2325	0.159	

TABLE IV

Run	Time interval, min.	Gas rate, gm-atoms/min. per liter soln.	Run	Time interval, min.	Gas rate, gm-atoms/min. per liter soln.
1	221–241	1.55×10^{-4}	11	35–53	2.32×10^{-4}
	322–352	1.29		147–180	1.87
2	25–37	2.83	12	204–240	0.80
	90–111	2.16		335–363	0.66
3	19–35½	3.75	13	16–30	4.21
	78–94	3.42		76–91	3.55
				223–244	2.76
4	131–151	2.97			
	295–315	2.22	14	236–276	1.32
				304–323	1.27
5	35–45	6.13			
	182–197	3.77	15	274–295	0.45
6	285–330	0.32			
7	164–187	2.30			
	323–343	1.68			
8	82–117	0.87			
	196–236	0.71			
9	215–313	0.81			
10	96–116	2.55			
	160–180	2.56			

TABLE V

Run	Ionic strength	Temp., °C.	Time, min.	(ClO_2^-), *M*	(OCN^-), *M*	(NaOH), *M*
18	2.2	98	0	0.311	0.300	0.191
			34	0.308		
			240	0.251		
			310	0.220		

DISCUSSION OF RESULTS

Although the reaction approximates to

$$2OCN^- + 3OCl^- + 2OH^- \rightarrow 2CO_3^{--} + 3Cl^- + N_2 + H_2O$$

the amounts of cyanate and hypochlorite disappearing do not agree at all closely with this equation. In general more hypochlorite reacts than would be expected. Part of this must be due to the spontaneous decomposition of hypochlorite to chlorate and chloride, but this can only account for part of the discrepancy. The rate of gas evolution is also generally lower than is required by this equation. The most plausible secondary reaction is probably this:

$$OCN^- + 4OCl^- + 2OH^- \rightarrow CO_3^{--} + 4Cl^- + NO_3^- + H_2O.$$

Indeed it is difficult to suggest any other simple compound of nitrogen, except

possibly nitrous oxide, that might reasonably appear in this reaction mixture, and that would not be oxidized further.

The possibility of nitrous oxide formation was examined by collecting the gas evolved in a special gas burette, at the top of which was a coil of wire that could be heated. Hydrogen was added to the gas, and the wire heated to convert any nitrous oxide to nitrogen and water, and any oxygen to water. Then excess oxygen was added, and the wire was heated again. From the volumes at the various stages of this procedure it is possible to determine how much oxygen and nitrous oxide was present in the original gas. A little oxygen was detected (from decomposition of hypochlorite), but no nitrous oxide, within the experimental error.

Support for the suggestion that nitrate was formed was provided in two ways. First, the reaction mixture from a run with high hypochlorite was treated with enough 3% hydrogen peroxide to destroy the remaining hypochlorite ions. The mixture was then made very acid with concentrated sulphuric acid, and boiled. This removes any cyanate and the excess hydrogen peroxide, but the main object of this procedure was to remove chlorate. The boiling was continued until all chlorine and chlorine dioxide had boiled out of the solution, and addition of more sulphuric acid gave no further yellow color. After the solution was cooled, it was tested for nitrate by (i) the 'brown ring' test with ferrous sulphate and concentrated sulphuric acid, and (ii) 'nitron' acetate solution (7). The second test was applied both to the solution directly after it had been cooled, and to a portion of it which had been neutralized with sodium bicarbonate and then made just acid with acetic acid. Both tests gave a strong reaction for nitrate. Secondly, the amounts of substances appearing and disappearing agreed, roughly, with this secondary reaction. This second line of evidence is not very strong, as we are dealing with small differences in the analyses, or in the rates of gas evolution. The gas evolution itself can be easily measured, but there is always uncertainty as to whether this represents the true rate of reaction to nitrogen, because some of the gas can remain in solution. There was always some lag in the gas evolution at first (as has been noticed in other similar reactions), presumably because the concentration of gas in solution was building up. However after the reaction has been running for some time, the rate of gas evolution must be close to the true rate of reaction.

The calculations which were made to test the suggestion of this secondary reaction were as follows. Suppose we have $2a$ gm-molecules of cyanate reacting by the first reaction:

$$2OCN^- + 3OCl^- + 2OH^- \rightarrow 2CO_3^{--} + 3Cl^- + N_2 + H_2O \qquad \text{I}$$

and b gm-molecules by the second:

$$OCN^- + 4OCl^- + 2OH^- \rightarrow CO_3^{--} + 4Cl^- + NO_3^- + H_2O \qquad \text{II}$$

i.e. $2a$ gm-molecules of cyanate react with $3a$ gm-molecules of hypochlorite by the first reaction, etc. Then the total changes involved would be:

OCl^-: $-3a-4b$; OCN^-: $-2a-b$; total alkalinity: $-2a-2b$; nitrogen (gm-atoms): $2a$.

As the analyses for hypochlorite and cyanate were probably the most reliable,

values of a and b were obtained from them with the results in Table VI. Table VI also makes allowance for the amount of hypochlorite reacting to

TABLE VI

Run	Change in (OCl^-)	Change in (OCN^-)	Corrn. to OCl^-	Corrected change in (OCl^-)	a	b	b/a	Change in total alkalinity	
								Obs.	Calc.
1	0.1335	0.079	0.002	0.1315	0.0369	0.0052	0.14	0.11	0.084
2	0.228	0.110	0.008	0.220	0.0440	0.0220	0.50	0.109	0.132
3	0.291	0.140	0.019	0.272	0.0576	0.0248	0.43	0.134	0.165
4	0.205	0.123	0.001	0.204	0.0576	0.0078	0.14	0.094	0.131
5	0.279	0.214	0.001	0.278	0.115	(−.017)	—	0.30	0.23
6	0.049	0.026	0.004	0.045	0.0118	0.0024	0.20	0.030	0.028
7	0.168	0.096	0.0025	0.1655	0.0421	0.0086	0.20	0.091	0.101
8	0.0905	0.048	0.003	0.0875	0.0209	0.0062	0.29	0.049	0.054
9	0.0545	0.031	0.004	0.0505	0.0147	0.0016	0.11	0.048	0.033
10	0.139	0.080	0.0015	0.1375	0.0365	0.0070	0.19	0.072	0.087

give chlorate and chloride, which is calculated using a rate constant of 11×10^{-5} (gm-mol./l.)$^{-1}$ min.$^{-1}$. This constant is obtained from results of the writer (6) on this reaction. If x is the concentration of hypochlorite ion, then the rate of the reaction to chlorate is given by

$$-dx/dt = \tfrac{3}{2}k_1x^2$$

where k_1 is the rate constant. The value of x used was the average over the period investigated; that is, the correction used was:

$$\text{correction to hypochlorite used up} = \tfrac{3}{2}k_1[(x_{\text{initial}}+x_{\text{final}})/2]^2.\ (\text{time}).$$

The correction is small enough for this to be sufficiently accurate.

The agreement between the observed and calculated changes in the total alkalinity is very moderate; but we are, of course, dealing with small differences between large quantities. However the results in Table VI do provide some support for the suggested secondary reaction.

Table V shows that there is a slow reaction of chlorite and cyanate ions, but this is quite slow even at 98° C., and with 0.2 to 0.3 M chlorite present. As the chlorite present in hypochlorite from its decomposition is only about $\frac{1}{2}\%$ of the hypochlorite (6), the reaction of chlorite and cyanate is much too slow to contribute appreciably to the total reaction.

Turning to the gas rates, we see that these are generally lower than they would be if all the nitrogen from the cyanate were evolved as gas. If the alternative reaction is as suggested above, then the ratio of the rate of gas evolution (in gm-atoms/min.) to the rate of disappearance of cyanate would be $2a/(2a+b)$. Table VII(a) gives the results of such a comparison. The rates of cyanate disappearance were obtained by plotting the cyanate analyses and drawing smooth curves through them: the rates were read off these smoothed curves. Again there is moderate agreement, but on the whole the gas rates support the reaction proposed above.

TABLE VII

	Run	Time, min.	Gas rate, gm-atoms/min. per liter soln.	Cyanate rate, gm-mol./liter per min.	b/a	Gas rate calc.
(*a*)	1	221–241	1.55×10^{-4}	2.0×10^{-4}	0.14	1.85×10^{-4}
		322–352	1.29	1.5		1.4
	2	25–37	2.83	2.9	0.50	2.35
		90–111	2.16	2.5		2.0
	3	19–35½	3.75	4.0	0.43	3.3
		78–94	3.42			
	4	131–151	2.97	4.0	0.14	3.7
		295–315	2.22	2.4		2.25
	5	35–45	6.13	—	0	—
		182–197	3.77	4.5		4.5
	6	285–330	0.32	0.5	0.20	0.45
	7	164–187	2.30	2.5	0.20	2.3
		323–343	1.68	2.1		1.9
	8	82–117	0.87	1.4	0.29	1.2
		196–236	0.71	1.2		1.05
	9	215–313	0.81	0.8	0.11	0.75
	10	96–116	2.55	3.2	0.19	2.9
		160–180	2.56	3.0		2.7
(*b*)	11	35–53	2.32	3.4	0	3.4
		147–180	1.87			
	12	204–240	0.80	1.5	0.21	1.35
		335–363	0.66			
	13	16–30	4.21	4.9	0.58	3.8
		76–91	3.55	4.6		3.5
		223–244	2.76	4.2		3.2
	14	235–276	1.32	1.45	0.28	1.25
		304–323	1.27			
	15	274–295	0.45	0.75	0.27	0.65

The values of b/a are of some interest. Many of these are in the neighborhood of 0.1 to 0.2, which means that the reaction to nitrate is a minor one but not negligible. In runs 2 and 3, when (ClO^-) was high, so was b/a; thus a high concentration of hypochlorite favors, as might be expected, the more extreme oxidation to nitrate. Conversely, when (OCN^-) was very high in run 5, the ratio b/a was diminished. The apparent negative value of b in this run is presumably due to experimental error. When the sodium hydroxide concentration is varied, b/a does not change in any systematic manner, so sodium hydroxide does not affect the relative chances of the reaction going to nitrogen or nitrate. Thus, so far as they go, these values of b/a are consistent with the suggested reactions.

If we now examine the rates of reaction in runs 1 to 10, we find from runs 1 to 3 that the rate is at least roughly proportional to (OCl^-), if the other concen-

trations are the same. Similarly from runs 1, 4, 5, and 6, the rate is roughly proportional to (OCN^-). If we obtain (OH^-) from the total alkalinity at the start, we find from runs 1, 7, 8, 9, and 10 that the rate is roughly inversely proportional to the hydroxide concentration. Then if x is the hypochlorite concentration (initially x_0); y is the cyanate concentration (initially y_0); and z is the hydroxide concentration (initially z_0); these results would mean that

$$-dx/dt = k^*xy/z$$

where k^* is a constant. If the first reaction (I above) is followed, we should write

$$-dx/dt = 3kxy/z$$

where k is the correct rate constant, since three hypochlorite ions are used up in the reaction, but presumably only one in the first stage. We also have:

$$2\,dx/dt = 3\,dy/dt = 3\,dz/dt.$$

These equations integrate to give:

$$[i] \qquad -kt = \frac{1}{2x_0-3y_0}\left[(\tfrac{2}{3}x_0-z_0)\ln\frac{x}{x_0}+(z_0-y_0)\ln\frac{(2x-2x_0+3y_0)}{3y_0}\right].$$

The best way to check this equation is to calculate kt for various values of x, and then to fit the experimental points to this curve for each run by taking some value of k. This was done (see Fig. 1), and in general an adequate fit was

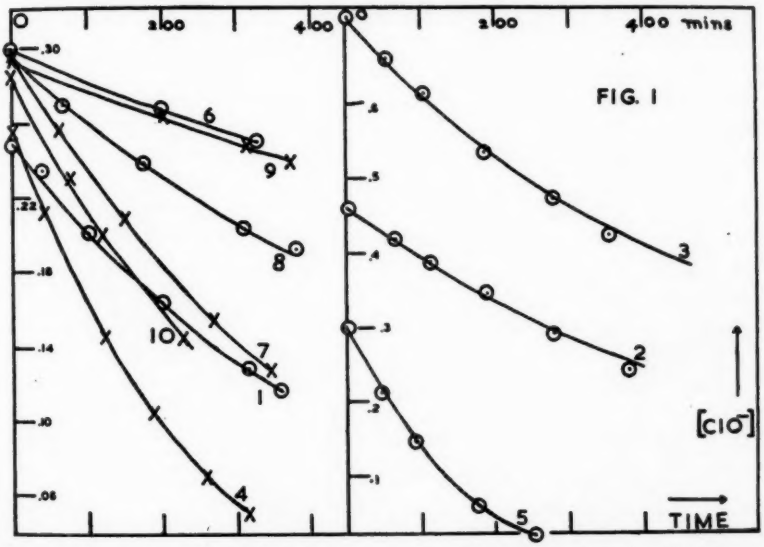

FIG. 1. Comparison of hypochlorite concentrations calculated by equation [i] with experimental points.

obtained. This supports the above rate equation, and gives the following values of k:

Run	1	2	3	4	5	6	7	8	9	10	
k	6.28	6.99	6.94	6.17	5.67	6.07	5.58	6.72	6.40	5.65	$\times 10^{-4}$ min.$^{-1}$

Before accepting these values of the constants, we must correct them for the effect of the decomposition of hypochlorite to chlorate, and for the effect of the secondary reaction. If the total reaction followed the second equation to nitrate and carbonate, the rate equations would be:

$$-dx/dt = 4kxy/z, \qquad dx/dt = 4\,dy/dt = 2\,dz/dt.$$

These give on integration:

$$[ii] \qquad -kt = \frac{1}{x_0-4y_0}\left[(\tfrac{1}{2}x_0-z_0)\ln\frac{x}{x_0}+(z_0-2y_0)\ln\frac{(x-x_0+4y_0)}{4y_0}\right].$$

This equation gives a curve only a little different from equation [i], if we take the best value of k to fit the experimental points. In runs 2 and 3 where the secondary reaction is the most important, equation [ii] gives a curve which deviates from the experimental points in the opposite direction from equation [i], so a combination of both gives an excellent fit.

In a more general way, if c is the chance that the second reaction will occur (i.e. $c = b/a$), and $(1-c)$ is the chance that the first reaction will occur; then $(3+c)$ hypochlorite ions, $(2-c)$ cyanate ions, and 2 hydroxide ions will be used up for each initial step. This leads to the more general equation:

$$[iii] \quad -kt = \frac{1}{(3+c)\,y_0-(2-c)\,x_0}\left[\left(z_0-\frac{2x_0}{3+c}\right)\ln\frac{x}{x_0} + \left(\frac{2y_0}{2-c}-z_0\right)\ln\frac{(3+c)\,y_0}{(3+c)\,y_0+(2-c)(x-x_0)}\right].$$

If $c = 0$ this reduces to equation [i], and if $c = 1$ it reduces to equation [ii]. It is assumed that reaction II has the same initial step as reaction I. The evidence for this is not very definite because reaction II is masked by the predominant reaction I. However it will be shown in the calculations which follow that in the runs (runs 2 and 3) when the second reaction is most important, the rate constant of reaction I is still in reasonable agreement with that found in other runs; nor is there any apparent relation between this rate constant and b/a. Also, in various runs values of b run roughly parallel to those for a, measured at the same time. For these reasons, and in the absence of definite evidence to the contrary, it seemed simplest to suppose that the two reactions had the same initial step.

Calculating k from equation [iii], we get:

Run	1	2	3	4	5	6	7	8	9	10	
k	5.94	5.27	5.58	5.90	5.67	5.53	5.17	6.04	6.14	5.27	$\times 10^{-4}$ min.$^{-1}$

The reaction to chlorate can be corrected for as follows. The amount of hypochlorite reacting in this way was calculated earlier in obtaining the b/a ratios. This was between $\frac{1}{2}$ and $8\frac{1}{2}\%$ of the total hypochlorite reacting; and it was assumed that the rate constants (which are proportional to the rate of disappearance of hypochlorite) would be too high by the same per cent, as obtained above from equation [iii]. As a result the corrected values were:

Run	1	2	3	4	5	6	7	8	9	10	
% corrn.	1.47	3.98	6.79	0.65	0.53	8.37	1.63	3.51	6.82	1.29	
k(corr.)	5.85	5.06	5.20	5.86	5.64	5.07	5.09	5.83	5.72	5.20	$\times 10^{-4}$ min.$^{-1}$

The mean value of k was 5.45×10^{-4}, with an average deviation of 0.33×10^{-4}. These values of k can be described as only moderately constant, but it is believed that this is due to the experimental errors in the analyses, and not to the selection of the wrong kinetic scheme. It will be noted that run 7, when a considerable quantity of sodium carbonate was added, gave the same constant, so presumably sodium carbonate and chloride do not catalyze the reaction, apart from their effect on the ionic strength.

We can apply similar calculations to the results in Tables II and III. The data that determine the relative extent of the two reactions are given in Table VIII. The values of b/a in Table VIII show much the same trends as

TABLE VIII

Run	Ionic strength	Temp., ° C.	Change in (OCl^-)	Change in (OCN^-)	Corrn. to OCl^-	Corrected change in (OCl^-)	a	b	b/a
11	1.7	65	0.2125	0.140	0.005	0.2075	0.0705	(−.001)	0
12	2.0	65	0.123	0.066	0.008	0.115	0.0298	0.0064	0.21
13	2.9	65	0.306	0.126	0.046	0.260	0.0488	0.0284	0.58
14	3.5	65	0.0995	0.054	0.0015	0.098	0.0236	0.0068	0.28
15	2.2	55	0.0775	0.036	0.002	0.0755	0.0185	0.0050	0.27
16	2.2	75	0.193	0.0945	0.003	0.0190	0.0398	0.0184	0.46
17	2.2	50	0.2355	0.128	0.0135	0.222	0.0580	0.0136	0.23

before. In run 13, for instance, the high hypochlorite concentration makes b/a high. The run at 75° C. seems to have a high value of b/a, and it is of course reasonable to suppose that temperature could alter the extent of these reactions. The numbers do give some indication that high temperature or ionic strength both increase b/a, but the results are too few for any firm conclusions to be drawn.

If we evaluate k precisely as before, the final corrected results are:

Run	11	12	13	14	15	16	17	
Temp.	65	65	65	65	55	75	50	° C.
Ionic strength	1.7	2.0	2.9	3.5	2.2	2.2	2.2	
k	4.22	4.85	5.52	6.20	1.89	16.8	1.00	$\times10^{-4}$ min.$^{-1}$

The results for run 13 cannot be very accurate because of the large correction for the reaction to chlorate. The values of k show an upward drift with ionic strength; the extent of the change is comparable with that found in similar reactions, e.g. that of hypochlorite going to chlorate.

If log k is plotted against $1/T$ in the usual way, a fairly straight line is obtained. The over-all (50° C. to 75° C.) value for the activation energy is 25.3 kcal./gm-mol. If we allow a little more weight to the value at 65° C., because it was more carefully measured, the activation energy is reduced a little: probably the best value is $25(\pm\frac{1}{2})$ kcal./gm-mol.

Comparison of the observed and calculated gas rates for runs 11 to 17 as given in Table VII(b) shows about the same degree of agreement as before. The calculated rates were obtained in the same way as in Table VII(a).

The next question is that of what one can conclude about the mechanism of this reaction. The kinetics suggest that the slow step is really a bimolecular reaction of HOCl and OCN^-. If k_t is the true rate constant of this reaction, then comparison with the rate equations used earlier shows that:

$$k_t(HOCl) = k(OCl^-)/(OH^-)$$

[iv] or

$$k_t = K_a.k/K_w$$

where K_a is the ionization constant of hypochlorous acid, and K_w is the ionization constant of water. At 65° C., k is 5.45×10^{-4} and K_w is 1.25×10^{-13} (2). There are no values of K_a reported for 65° C., but it is 3.8×10^{-8} at 27° C. (5). If we use the value of the heat of ionization given below, the calculated ionization constant at 65° C. is 8×10^{-8}. Hence k_t at 65° C. is 350 min.$^{-1}$ M.p.l.$^{-1}$, or 5.8 sec.$^{-1}$ M.p.l^{-1}. This is a fairly fast reaction, and the question arises whether this is consistent with the observed temperature dependence on the rate. By taking $-1/R \cdot d\ \ln/d(1/T)$ of both sides of equation [iv] we get:

$$E_t = E + q_a - q_w$$

where E_t is the true activation energy of the reaction of HOCl with OCN^-, and E is the apparent activation energy (25 kcal.) obtained above. q_a is the heat of the reaction:

$$H^+ + OCl^- \rightarrow HOCl + q_a$$

and q_w is the heat of recombination of the ions of water: 13.8 kcal. (1). There seem to be no direct values for the heat of ionization of hypochlorous acid, but data given by Latimer (3) make $q_a = 3.9$ kcal. Substituting these values, $E_t = 15.1$ kcal./gm-mol. This cannot be considered to be very accurate, chiefly because of uncertainty in q_a. If, as is usual, we suppose that k_t and E_t are related by $k_t = Ae^{-E_t/RT}$, then the value of log A is 10.5. This is quite a plausible value for A; and, while no great accuracy can be claimed, it seems safe to conclude that there is no inconsistency in the observed rates and temperature dependence.

Finally, if the first step is a reaction of hypochlorous acid and cyanate ions, it is natural to consider what might be the intermediate products of such a reaction. Unfortunately these products are not obvious, and what follows is largely speculation. However it is perhaps reasonable to assume that chloride ions are formed, as in other oxidations by hypochlorous acid. The reaction might then be:

$$HOCl + OCN^- + H_2O \rightarrow Cl^- + HO_2CNHOH$$

followed by further rapid oxidation. Alternatively, HOCl might possibly add across a double or triple bond in a cyanate ion, to give the rather awkward looking ion $(O{=}C.OH{-}NCl)^-$ which would doubtless rearrange to $(O_2C{-}NHCl)^-$. The first suggestion looks rather more plausible. At least one can conclude that such products are not wholly ridiculous, and the kinetics presumably show that some such reaction really occurs.

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ÉTUDES SUR LA SYNTHÈSE DE L'HYDROXYPROLINE À PARTIR DE DÉRIVÉS DE L'ACIDE 2-AMINO-4-PENTÈNOÏQUE[1]

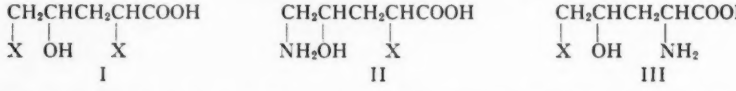
PAR ROGER GAUDRY, LOUIS BERLINGUET, ANDRÉ LANGIS ET GÉRARD PARIS

ABSTRACT

A systematic investigation of the synthesis of 4-hydroxy-DL-proline and 2-amino-4-dihydroxyvaleric acid has been made, starting from the following derivatives of 2-amino-4-pentenoic acid: ethyl allylacetamidomalonate, ethyl allylacetamidocyanoacetate, 2-phthalimidopentenoic acid, allylacetamidomalonic acid, acetylallylglycine, 5-allylhydantoin, and 3-phenyl-5-allylhydantoin. Chlorine or bromine was added to the double bond of these compounds, and the reaction products were either derivatives of 5-halogenated-4-valerolactones or derivatives of 4,5-dihalogenated pentanoic acids, depending on whether the carboxyl group of the pentanoic acid was free or not when the halogenation reaction was carried out. Acid hydrolysis followed by treatment with barium hydroxide always gave mixtures, in different ratio, of 4-hydroxy-DL-proline and 2-amino-4,5-dihydroxyvaleric acid which were analyzed and isolated as the copper salts. In the case of 5-(2,3-dibromopropyl)hydantoin and 3-phenyl-5-(2,3-dibromopropyl)hydantoin, no cyclization could be obtained.

INTRODUCTION

Nous avons entrepris d'étudier systématiquement la synthèse de la 4-hydroxyproline dans le but d'élaborer une synthèse permettant l'obtention facile de grandes quantités de cet acide aminé naturel. On peut ramener à trois méthodes générales les synthèses de l'hydroxyproline publiées à date. La première consiste à cycliser par l'ammoniac un dérivé 2,5-dihalogéné de l'acide 4-hydroxyvalérianique (I), donc un produit ne contenant pas d'azote. La deuxième consiste à cycliser par une base un dérivé 2-halogéné de l'acide 4-hydroxy-5-aminovalérianique (II), c'est-à-dire une substance renfermant de l'azote aminé en position 5. La troisième consiste au contraire à cycliser par une base un dérivé 5-halogéné de l'acide 2-amino-4-hydroxyvalérianique (III), renfermant par conséquent de l'azote aminé en position 2.



I II III

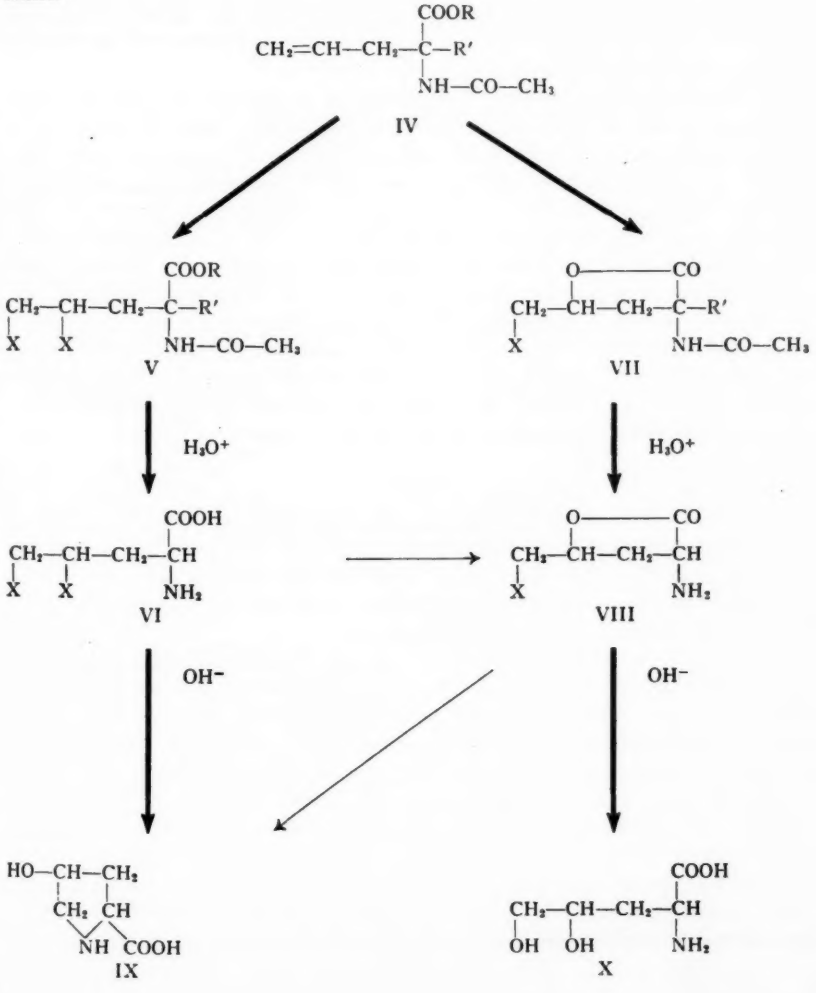
Dans une publication antérieure (5), nous avons discuté de la synthèse de l'hydroxyproline à partir de dérivés 2,5-dihalogénés de l'acide 4-hydroxyvalérianique (I) et 2-halogénés de l'acide 4-hydroxy-5-aminovalérianique (II). Nous exposons maintenant les résultats de nos travaux sur la préparation de l'hydroxyproline en passant par les dérivés 5-halogénés de l'acide 2-amino-4-hydroxyvalérianique (III).

On trouve dans la littérature quatre synthèses de l'hydroxyproline qui utilisent l'acide 2-amino-4-hydroxy-5-halogénovalérianique comme intermédiaire: celle de Fischer et Krämer (3), Hammarsten (6), McIlwain et Richardson (9) ainsi que Feofilaktov et Onishchenko (2). Mais celle de Hammarsten est la seule à utiliser comme produit de départ un dérivé de l'acide 2-amino-4-pentènoïque: l'acide allylhippurique ou acide 2-benzamido-4-pentènoïque.

[1]*Manuscrit reçu pour la première fois le 18 août 1955, et sous forme revisée le 29 décembre 1955. Contribution du Département de Biochimie de la Faculté de Médecine, Université Laval, Québec, P.Q.*

Nous avons repris la méthode de Hammarsten, et nous l'avons appliquée, avec modifications appropriées, à plusieurs types de dérivés de l'acide 2-amino-4-pentènoïque, soit: l'allylacétamidomalonate d'éthyle (IV, $R = C_2H_5$, $R' = CO_2C_2H_5$), l'allylacétamidocyanoacétate d'éthyle (IV, $R = C_2H_5$, $R' = CN$), l'acide 2-phtalimido-4-pentènoïque (IV, $R = R' = H$, $-N(CO)_2C_6H_4$ remplace $-NHCOCH_3$), l'acide allylacétamidomalonique (IV, $R = H$, $R' = COOH$), l'acétylallylglycine (IV, $R = R' = H$), la 5-allylhydantoïne ainsi que la 3-phényl-5-allylhydantoïne.

Par addition de chlore ou de brome à l'allylacétamidomalonate d'éthyle (IV, $R = C_2H_5$, $R' = CO_2C_2H_5$), on obtient les esters correspondants à l'état cristallin: le dichloré avec 18% de rendement; le dibromé avec 50% de rendement.



Par addition de chlore ou de brome à l'allylacétamidocyanoacétate d'éthyle (IV, $R = C_2H_5$, $R' = CN$), on obtient les esters correspondants à l'état cristallin mais qui sont beaucoup plus faciles à cristalliser et à isoler que les produits analogues provenant de l'allylacétamidomalonate d'éthyle (IV, $R = C_2H_5$, $R' = CO_2C_2H_5$). L'ester dichloré est obtenu avec un rendement de 70% et l'ester dibromé avec un rendement de 75%.

L'addition d'halogènes sur l'acide 2-phtalimido-4-pentènoïque (IV, $R = R' = H$, $—N(CO)_2C_6H_4$ remplace $—NHCOCH_3$), sur l'acide allylacétamidomalonique (IV, $R = H$, $R' = COOH$) et sur l'acétylallylglycine (IV, $R = R' = H$) donne surtout les dérivés de la 2-amino-4-valérolactone-5-halogénée, les acides dihalogénés se cyclisant avec le groupement carboxyle libre.

L'hydrolyse acide des dérivés 4,5-dihalogénés donne surtout l'acide 2-aminovalérianique-4,5-dihalogéné. Et par chauffage en milieu alcalin, on obtient la 4-hydroxyproline (IX) avec de l'acide 2-amino-4,5-dihydroxyvalérianique (X) comme sous-produit.

Par contre, l'hydrolyse acide des dérivés de la 2-amino-4-valérolactone-5-halogénée (VII), suivie de chauffage en milieu alcalin, mène de préférence à l'acide 2-amino-4,5-dihydroxyvalérianique (X), avec la 4-hydroxyproline (IX) comme produit secondaire. On peut expliquer ces résultats en considérant que, par traitement alcalin, l'anneau des lactones doit s'ouvrir avant que l'anneau de pyrrolidine puisse se former. Il y a alors réactions compétitives entre l'hydrolyse de la lactone et le remplacement de l'atome d'halogène en position 5 par une fonction alcool. Lorsque l'atome d'halogène est un atome de brome, il semble que dans la majorité des cas, il soit en bonne partie éliminé avant l'ouverture de l'anneau de l'acide 2-amino-4,5-dihydroxyvalérianique. Lorsque l'atome d'halogène est l'atome de chlore, il réagit lentement avec l'alcali. Dans ce cas, il peut se former une quantité appréciable d'acide 2-amino-4-hydroxy-5-chlorovalérianique qui se cyclise aussitôt en hydroxyproline.

En conséquence, les lactones bromées mènent presque exclusivement à l'acide dihydroxylé tandis que les lactones chlorées mènent à des mélanges d'hydroxyproline et d'acide dihydroxylé.

Les essais de séparation de ces deux acides aminés au moyen des picrates (7), des sels de reineke (10), des chlorhydrates, ainsi que la séparation sur des colonnes de résines se sont avérés infructueux.

Nous avons réussi à effectuer la séparation des deux acides aminés par cristallisation fractionnée des sels de cuivre de l'hydroxyproline et de l'acide dihydroxylé dans l'eau puis dans l'alcool méthylique anhydre. Ainsi nous avons pu isoler le sel de cuivre de l'acide dihydroxylé, qui jusqu'alors n'avait jamais été isolé de façon certaine (3,6) et le sel de cuivre de l'hydroxyproline. Nous n'avons toutefois pas tenté la séparation des formes "allo" et "DL".

Quant à l'étude des meilleures conditions expérimentales pour ces synthèses, nous avons déterminé les rendements des deux acides aminés, hydroxyproline et acide 2-amino-4,5-dihydroxyvalérianique, par dosage chimique au moyen respectivement des méthodes de McFarlane et Guest (8) et de N. W. Rees (11). Cette dernière méthode avait été tout d'abord employée pour doser la sérine

et la thréonine. Nous l'avons adoptée au dosage de l'acide 4,5-dihydroxylé avec tout autant de succès.

Ces analyses ont été effectuées sur les liqueurs provenant de l'hydrolyse acide et du traitement alcalin des produits dihalogénés (Tableau I). Elles ont aussi été déterminées sur les solutions obtenues après halogénation, hydrolyse acide, traitement alcalin des dérivés de l'acide 2-amino-4-pentènoïque (Tableau II). Les différences entre les valeurs des deux tableaux, proviennent évidemment des difficultés rencontrées lors de l'isolement des produits intermédiaires.

TABLEAU I

RENDEMENTS EN HYDROXYPROLINE ET EN ACIDE 2-AMINO-4,5-DIHYDROXYVALÉRIANIQUE PAR ANALYSE DE LA SOLUTION APRÈS HYDROLYSE ACIDE ET CYCLISATION ALCALINE DU PRODUIT DIHALOGÉNÉ ISOLÉ

	Rendement en %		
	Hydroxyproline	Acide dihydroxylé	Total
Ester éthylique de l'acide 2-carbéthoxy-2-acétamido-4,5-dichlorovalérianique	69	13	82
Ester éthylique de l'acide 2-carbéthoxy-2-acétamido-4,5-dibromovalérianique	57	33	90
Ester éthylique de l'acide 2-acétamido-2-cyano-4,5-dichlorovalérianique	79	17	96
Ester éthylique de l'acide 2-acétamido-2-cyano-4,5-dibromovalérianique, p.f. 131° C.	25	63	88
2-Phtalimido-5 chlorovalérolactone	27	38	65
2-Phtalimido-5-bromovalérolactone	7	65	72

TABLEAU II

RENDEMENTS EN HYDROXYPROLINE ET EN ACIDE 2-AMINO-4,5-DIHYDROXYVALÉRIANIQUE PAR ANALYSE DE LA SOLUTION APRÈS HALOGÉNATION, HYDROLYSE ACIDE ET CYCLISATION ALCALINE DES DÉRIVÉS DE L'ACIDE 2-AMINO-4-PENTÈNOÏQUE

		Rendement en %		
		Hydroxyproline	Acide dihydroxylé	Total
Allylacétamidomalonate d'éthyle plus:	Cl_2 à 0° C.	74	13	87
	Cl_2 à 25° C.	67	23	90
	SO_2Cl_2 à 25° C.	78	17	95
	Br_2 à 0° C.	13	51	64
	Br_2 à 25° C.	28	49	77
Allylacétamidocyanoacétate d'éthyle plus:	Cl_2 à 25° C.	52	18	70
	SO_2Cl_2 à 25° C.	64	24	88
	Br_2 à 25° C.	48	37	85
Acide 2-phtalimido-4-pentènoïque plus:	Cl_2 à 25° C.	21	57	78
	Br_2 à 25° C.	24	48	72

Dans une publication récente portant sur la proline, l'un de nous (4) a démontré qu'on peut cycliser la 5-(3-chloropropyl)hydantoïne en l'hydantoïne de la proline, et que la 3-phenyl-5-(3-bromopropyl)hydantoïne se cyclise spontanément en phénylhydantoïne de la proline. Nous avons voulu appliquer ces réactions à l'hydroxyproline. Nous avons préparé la 5-(2,3-dibromopropyl) hydantoïne, ainsi que la 3-phenyl-5-(2,3-dibromopropyl)hydantoïne. Mais tous nos essais de cyclisation de ces hydantoïnes en dérivés de l'hydroxyproline se sont avérés infructueux.

Il ressort de ces travaux que la préparation des acides 2-aminovalérianique-4,5-dihalogénés, conduisant à l'hydroxyproline ne peut se faire sans qu'une certaine partie de l'acide ne se cyclise en 2-amino-4-valérolactone-5-halogénée, laquelle lactone, par traitement alcalin, donne surtout l'acide 2-amino-4,5-dihydroxyvalérianique. Les quantités d'hydroxyproline obtenues, quoique élevées, sont toujours accompagnées de l'acide 2-amino-4,5-dihydroxyvalérianique qu'on peut fort heureusement séparer par la méthode décrite plus haut.

PARTIE EXPÉRIMENTALE

Allylacétamidomalonate d'éthyle (IV, $R = C_2H_5$, $R' = CO_2C_2H_5$)

Dans un ballon à 3 cols de 300 ml. muni d'un agitateur, d'un réfrigérant surmonté d'un tube de chlorure de calcium et d'un entonnoir à décantation, on place 100 ml. d'alcool anhydre dans lequel on dissout du sodium (4.6 g., 0.20 mole). On ajoute ensuite de l'acétamidomalonate d'éthyle (43.4 g., 0.20 mole). Après environ une demi-heure, on laisse tomber goutte à goutte du bromure d'allyle (28 g., 0.23 mole) en maintenant la température à 25° C. L'addition terminée on chauffe la solution à 40° C. pendant 20 h. L'alcool est évaporé sous pression réduite, puis on dissout le résidu dans 10 ml. d'eau distillée. Après avoir acidifié la solution avec de l'acide sulfurique, on extrait l'allylacétamidomalonate d'éthyle à l'éther. On sèche la solution éthérée sur du sulfate de sodium anhydre. L'éther est ensuite évaporé sous pression réduite et le produit de condensation cristallise lentement. On le recristallise de l'alcool éthylique aqueux. Rendement: 37 g., 72%. P. f.* 46° C., Litt.: 46° C. (1). Calculé pour $C_{12}H_{19}O_5N$: N, 5.45%. Trouvé: N, 5.41%.

Lorsqu'on substitue le chlorure d'allyle au bromure d'allyle, le rendement est de 45%.

Ester éthylique de l'acide 2-carbéthoxy-2-acétamido-4,5-dichlorovalérianique (V, $X = Cl$, $R = C_2H_5$, $R' = CO_2C_2H_5$)

Dans un ballon à large col de 300 ml., on dissout de l'allylacétamidomalonate d'éthyle (9 g., 0.035 mole) dans 50 ml. de chloroforme. On refroidit la solution à 10° C. et on y fait barbotter du chlore jusqu'à addition du poids théorique. On évapore le solvant sous pression réduite et on dissout le résidu gommeux obtenu dans 25 ml. d'alcool éthylique. On verse la solution alcoolique dans de l'eau glacée. Le produit dichloré cristallise. Après recristallisation de l'alcool dilué le rendement est de: 2 g., 18%. P. f. 54–61° C. Calculé pour $C_{12}H_{19}O_5NCl_2$: N, 4.28%; Cl, 21.4%. Trouvé: N, 4.25%; Cl, 22.2%. On peut aussi faire l'addition de chlore au moyen de chlorure de sulfuryle.

**Les points de fusion ne sont pas corrigés.*

Ester éthylique de l'acide 2-carbéthoxy-2-acétamido-4,5-dibromovalérianique (V, $X = Br$, $R = C_2H_5$, $R' = CO_2C_2H_5$)

On place de l'allylacétamidomalonate d'éthyle (20 g., 0.078 mole) et 50 ml. de chloroforme dans un ballon à large col de 500 ml. Après dissolution, on ajoute goutte à goutte du brome (15 g., 0.094 mole) dissous dans 10 ml. de chloroforme, en maintenant la température à 20° C. Le solvant est ensuite évaporé. On se débarrasse de l'excès de brome en utilisant une solution aqueuse de bisulfite de sodium. On dissout le résidu gommeux dans 25 ml. d'alcool éthylique et on verse la solution dans de l'eau glacée. Le produit dibromé cristallise. On le recristallise de l'alcool dilué. Rendement: 17 g., 50%. P. f. 85° C. Calculé pour $C_{12}H_{19}O_5NBr_2$: N, 3.35%; Br, 38.3%. Trouvé: N, 3.47%; Br, 39.3%.

Hydrolyse et cyclisation alcaline

(*a*) *A partir de l'ester éthylique de l'acide 2-carbéthoxy-2-acétamido-4,5-dichlorovalérianique*

L'ester éthylique de l'acide 2-carbéthoxy-2-acétamido-4,5-dichlorovalérianique (1 g., 0.0031 mole) est dissous dans 15 ml. d'acide acétique glacial, puis hydrolysé par 25 ml. d'acide chlorhydrique concentré pendant quatre heures. On évapore la solution à sec sous pression réduite et on reprend le résidu à l'eau. On ajoute un excès d'hydroxyde de baryum (0.8 g., 0.0047 mole) et l'on chauffe à reflux pendant 16 h. L'hydroxyde de baryum est précipité avec du carbonate d'ammonium. Après avoir filtré le carbonate de baryum, la solution est évaporée à sec sous pression réduite. Le résidu est repris à l'eau, traité au noir animal, filtré et analysé par la méthode de McFarlane and Guest (8) pour l'hydroxyproline et la méthode de M. W. Rees (11) pour l'acide 2-amino-4,5-dihydroxyvalérianique. Le dosage de la solution donne les rendements suivants: hydroxyproline, 69%; acide 2-amino-4,5-dihydroxyvalérianique, 13%.

Lorsque l'on additionne à 25° C. du chlorure de sulfuryle (2.7 g., 0.020 mole) à l'allylacétamidomalonate d'éthyle (5 g., 0.019 mole) sans isoler le produit dichloré, l'on obtient, après hydrolyse et cyclisation alcaline, les rendements suivants calculés par analyse à partir de l'allylacétamidomalonate d'éthyle: hydroxyproline, 78%; acide 2-amino-4,5-dihydroxyvalérianique, 17%.

(*b*) *A partir de l'ester éthylique de l'acide 2-carbéthoxy-2-acétamido-4,5-dibromovalérianique*

En procédant de la même façon l'ester éthylique de l'acide 2-carbéthoxy-2-acétamido-4,5-dibromovalérianique (1 g., 0.0024 mole) donne par hydrolyse et cyclisation alcaline les rendements suivants calculés par analyse: hydroxyproline, 57%; acide 2-amino-4,5-dihydroxyvalérianique, 33%.

Lorsque l'on additionne à 25° C. du brome (3.6 g., 0.0225 mole) à l'allylacétamidomalonate d'éthyle (5 g., 0.0195 mole) sans isoler le produit dibromé, l'on obtient après hydrolyse et cyclisation alcaline les rendements suivants calculés par analyse à partir de l'allylacétamidomalonate d'éthyle: hydroxyproline, 28%; acide 2-amino-4,5-dihydroxyvalérianique, 49%.

Acétamidocyanoacétate d'éthyle

L'acétamidocyanoacétate d'éthyle a été préparé par la méthode de l'hydrosulfite (12). L'isonitrosocyanoacétate d'éthyle formé par l'action de l'acide nitreux sur le cyanoacétate d'éthyle n'a pas été isolé mais immédiatement réduit par l'hydrosulfite de sodium en présence d'anhydride acétique. Rendement: 51%. P. f. 129° C. Le produit est recristallisé de l'acide acétique 15%.

Allylacétamidocyanoacétate d'éthyle (*IV*, $R = C_2H_5$, $R' = CN$)

On place 75 ml. d'alcool anhydre dans un ballon à 3 cols muni d'un agitateur et d'un réfrigérant surmonté d'un tube de chlorure de calcium. On y dissout du sodium (4.6 g., 0.2 mole), puis on refroidit la solution à 10–15° C. On ajoute de l'acétamidocyanoacétate d'éthyle (34 g., 0.2 mole). Après 15 min. on élève la température à 30–35° C. et on laisse tomber goutte à goutte du chlorure d'allyle (25 g., 0.33 mole). L'addition du chlorure terminée, on élève la température à 60–65° C. et l'on chauffe ainsi la solution pendant huit heures. On verse la solution dans un mélange d'eau et de glace et on obtient des cristaux d'allylacétamidocyanoacétate d'éthyle que l'on filtre et que l'on lave à l'eau froide. Rendement: 39 g., 93%. P. f. 89° C., Litt. 89° C. (1). Calculé pour $C_{10}H_{14}O_3N_2$: N, 13.3%. Trouvé: N, 12.9%. En substituant le bromure d'allyle, le rendement est à peu près le même.

Ester éthylique de l'acide 2-acétamido-2-cyano-4,5-dichlorovalérianique
(*V*, $X = Cl$, $R = C_2H_5$, $R' = CN$)

L'addition de chlore gazeux sur l'allylacétamidocyanoacétate d'éthyle (25 g., 0.12 mole) dissous dans le chloroforme à 10° C. suivant la méthode décrite pour l'ester éthylique de l'acide 2-carbéthoxy-2-acétamido-4,5-dichlorovalérianique donne le produit dichloré avec un rendement de: 25 g., 70%. P. f. 100–105° C. Calculé pour $C_{10}H_{14}O_3N_2Cl_2$: N, 10.27%; Cl, 25%. Trouvé: N, 9.80%; Cl, 26.1%.

Ester éthylique de l'acide 2-acétamido-2-cyano-4,5-dibromovalérianique
(*V*, $X = Br$, $R = C_2H_5$, $R' = CN$)

De la même façon que pour l'ester éthylique de l'acide 2-carbéthoxy-2-acétamido-4,5-dibromovalérianique, l'addition de brome (16 g., 0.1 mole) à l'allylacétamidocyanoacétate d'éthyle (20 g., 0.095 mole) à 20–25° C. dans le chloroforme donne 26 g. de produit. Rendement: 75%. P. f. 114–115° C. Calculé pour $C_{10}H_{14}O_3N_2Br_2$: N, 7.56%; Br, 43.3%. Trouvé: N, 7.56%; Br, 43.2%.

Le produit isolé contient un mélange des deux isomères possibles. Si l'on fait la bromuration à −5°–0° C. le produit dibromé fond à 131° C. Si par ailleurs la bromuration est effectuée à 40–45° C. l'isomère dibromé obtenu fond à 98–99° C.

5-Bromo-2-acétamido-2-cyano-4-valérolactone (*VII*, $X = Br$, $R' = CN$)

L'ester éthylique de l'acide 2-cyano-2-acétamido-4,5-dibromovalérianique (7.4 g., 0.02 mole) est dissous dans 100 ml. d'alcool absolu bouillant. On ajoute un équivalent de potasse alcoolique (1.12 g., 0.02 mole) puis l'on chauffe le

mélange à reflux pendant quatre heures. On laisse refroidir et on filtre le précipité de bromure de potassium. On évapore le solvant. On reprend le résidu à l'éther pour enlever les dernières traces de bromure et on filtre. L'éther est évaporé et l'on obtient comme résidu une huile blanche difficile à cristalliser. Poids: 1 g. P. f. 85° C. Calculé pour $C_8H_9O_3N_2Br$: N, 10.7%; Br, 30.6%. Trouvé: N, 10.9%; Br, 30.1%.

Hydrolyse et cyclisation alcaline

(*a*) *A partir de l'ester éthylique de l'acide 2-acétamido-2-cyano-4,5-dichlorovalérianique*

L'ester éthylique de l'acide 2-acétamido-2-cyano-4,5-dichlorovalérianique (2 g., 0.0071 mole) hydrolysé et cyclisé de la même façon que l'ester éthylique de l'acide 2-carbéthoxy-2-acétamido-4,5-dichlorovalérianique donne les rendements suivants obtenus par analyse: hydroxyproline, 79%; acide 2-amino-4,5-dihydroxyvalérianique, 17%.

Lorsque l'on additionne à 25° C. du chlorure de sulfuryle (2 g., 0.015 mole) à l'allylacétamidocyanoacétate d'éthyle (2 g., 0.010 mole) sans isoler le produit dichloré, l'on obtient après hydrolyse et cyclisation alcaline les rendements suivants calculés à partir de l'allylacétamidocyanoacétate d'éthyle et obtenus par analyse: hydroxyproline, 64%; acide 2-amino-4,5-dihydroxyvalérianique, 24%.

(*b*) *A partir de l'ester éthylique de l'acide 2-acétamido-2-cyano-4,5-dibromovalérianique*

L'ester éthylique de l'acide 2-acétamido-2-cyano-4,5-dibromovalérianique (2 g., 0.005 mole), p.f. 131° C., en procédant de la même façon qu'à partir de l'ester éthylique de l'acide 2-carbéthoxy-2-acétamido-4,5-dichlorovalérianique, donne les rendements suivants obtenus par analyse: hydroxyproline, 25%; acide 2-amino-4,5-dihydroxyvalérianique, 63%.

Par addition de brome à 25° C. (2 g., 0.0125 mole) sur l'allylacétamidocyanoacétate d'éthyle (2 g., 0.0095 mole) et sans isoler le produit dibromé, on obtient par analyse, après hydrolyse et cyclisation alcaline, les rendements suivants: hydroxyproline, 48%; acide 2-amino-4,5-dihydroxyvalérianique, 37%.

Allylglycine

Dans un ballon de 300 ml., on place de l'allylacétamidocyanoacétate d'éthyle (10 g., 0.047 mole). On y ajoute 150 ml. d'eau distillée et 10 ml. d'acide sulfurique concentré. Après avoir chauffé la solution à reflux pendant quatre heures, on la neutralise avec de l'hydroxyde de baryum. On filtre le précipité de sulfate de baryum et on se débarrasse de l'excès d'hydroxyde de baryum en le faisant réagir avec un excès de carbonate d'ammonium. On filtre et on évapore la solution à sec sous pression réduite. On reprend le précipité avec 25 ml. d'eau distillée. On décolore la solution au noir animal et on précipite l'acide aminé en ajoutant 75 ml. d'alcool éthylique à la solution aqueuse. On filtre l'allylglycine et on sèche à l'air. Rendement: 2.5 g., 46%. P. f. 246–248° C. Calculé pour $C_5H_9O_2N$: N, 12.16%. Trouvé: N, 12.13%.

Acide 2-phtalimido-4-pentènoïque (*IV*, $R=R'=H$, $-N(CO)_2C_6H_4$ *remplace* $-NHCOCH_3$)

Dans un ballon de 100 ml., on place un mélange intime d'allylglycine (11.5 g., 0.1 mole) et d'anhydride phtalique (14.8 g., 0.1 mole) que l'on chauffe à une température de 180° C. pendant 10 min. Après avoir refroidi le produit, on le cristallise de l'alcool éthylique aqueux à 40% (125 ml.). Rendement: 21 g., 85%. P. f. 120–121° C. Calculé pour $C_{13}H_{11}O_4N$: N, 5.73%. Trouvé: N, 5.89%.

2-Phtalimido-5-chloro-4-valérolactone (*VI*, $X=Cl$, $R'=H$, $-N(CO)_2C_6H_4$ *remplace* $-NHCOCH_3$)

Dans un ballon à large col de 50 ml., on place de l'acide 2-phtalimido-4-pentènoïque (7.35 g., 0.03 mole) que l'on dissout dans 100 ml. d'acide acétique glacial. On y ajoute goutte à goutte, à la température de la chambre, du chlorure de sulfuryle (5 g., 0.037 mole). Le produit de la réaction cristallise de la solution à mesure qu'il se forme. On le recristallise de l'acétone. Rendement: 1.7 g., 20.5%. P. f. 187–188° C. Calculé pour $C_{13}H_{10}O_4NCl$: N, 5.02%; Cl, 12.71%. Trouvé: N, 5.10%; Cl, 12.68%.

2-Phtalimido-5-bromo-4-valérolactone (*VII*, $X=Br$, $R'=H$, $-N(CO)_2C_6H_4$ *remplace* $-NHCOCH_3$)

On dissout de l'acide phtalimidopentènoïque (24.5 g., 0.1 mole) dans 200 ml. d'acide acétique glacial. On ajoute goutte à goutte du brome (12.6 g., 0.079 mole) dans 50 ml. de tétrachlorure de carbone, en maintenant une bonne agitation. Le produit de la réaction cristallise de la solution à mesure qu'il se forme. On le recristallise d'un peu d'acétone. Rendement: 10,2 g., 31%. P. f. 189–190° C. Calculé pour $C_{13}H_{10}O_4NBr$: N, 4.32%; Br, 24.3%. Trouvé: N, 4.33%; Br, 24.4%.

Hydrolyse et cyclisation alcaline

(*a*) *A partir de la 2-phtalimido-5-chloro-4-valérolactone*

La 2-phtalimido-5-chloro-4-valérolactone (1 g., 0.0035 mole) est dissoute dans un mélange de 20 ml. d'acide acétique glacial et de 20 ml. d'acide chlorhydrique concentré. On chauffe à reflux pendant quatre heures, on évapore à sec, on reprend à l'eau et on filtre l'acide phtalique. On neutralise le filtrat avec de l'hydroxyde de baryum et on ajoute un excès d'hydroxyde de baryum 1.8 g. (0.0105 mole). On chauffe à reflux pendant six heures, on précipite le baryum au moyen d'un excès de carbonate d'ammonium et on filtre le précipité de carbonate de baryum. La solution est évaporée à sec sous pression réduite. Le résidu est repris à l'eau, filtré et analysé. Le dosage de la solution donne les rendements suivants: hydroxyproline, 27%; acide 2-amino-4,5-dihydroxyvalérianique, 38%.

Lorsqu'on additionne du chlorure de sulfuryle (2 g., 0.015 mole) à l'acide 2-phtalimido-4-pentènoïque (3 g., 0.012 mole) sans isoler le produit chloré, l'on obtient par analyse après hydrolyse et cyclisation alcaline les rendements suivants: hydroxyproline, 21%; acide 2-amino-4,5-dihydroxyvalérianique, 57%.

(*b*) *A partir de la 2-phtalimido-5-bromo-4-valérolactone*

De la même façon, par hydrolyse et cyclisation de la 2-phtalimido-5-bromo-4-valérolactone (1 g., 0.0031 mole), l'analyse donne les rendements suivants: hydroxyproline, 7%; acide 2-amino-4,5-dihydroxyvalérianique, 65%.

L'addition de brome (2 g., 0.012 mole) sur l'acide 2-phtalimido-4-pentènoïque (3 g., 0.012 mole), sans isoler le produit bromé, donne par analyse après hydrolyse et cyclisation alcaline les rendements suivants: hydroxyproline, 24%; acide 2-amino-4,5-dihydroxyvalérianique, 48%.

Acide allylacétamidomalonique (*IV*, $R=H$, $R'=COOH$)

On dissout de l'allylacétamidomalonate d'éthyle (25.7 g., 0.1 mole) dans de l'hydroxyde de sodium (11.2 g., 0.28 mole) solubilisé dans 50 ml. d'eau distillée et 50 ml. d'alcool éthylique. On laisse la solution à la température de la chambre pendant 24 h. en maintenant une bonne agitation. A la solution, on ajoute de la glace ainsi que 200 ml. d'acide chlorhydrique concentré. On évapore l'alcool sous pression réduite en ne chauffant que légèrement. L'acide allylacétamidomalonique cristallise lorsqu'on refroidit la solution. Rendement: 13.2 g., 65%. P. f. 100–103° C. Calculé pour $C_8H_{11}O_5N$: N, 6.96%. Trouvé: N, 7.00%.

Acétylallylglycine (*IV*, $R=R'=H$)

Dans un ballon de 300 ml., on place de l'acide allylacétamidomalonique (37 g., 0.185 mole) que l'on chauffe sur un bain de cire à une température de 125° C. jusqu'à ce que le dégagement de CO_2 cesse. Le produit est dissous dans 150 ml. d'eau distillée. On décolore la solution au noir animal et on évapore le solvant sous pression réduite. On dissout le résidu dans du chloroforme et on précipite l'acétylallylglycine avec de l'éther de pétrole. Rendement: 16.5 g., 57.0%. P. f. 112° C. Calculé pour $C_7H_{11}O_3N$: N, 8.92%. Trouvé: N, 8.97%.

Bromuration, hydrolyse et cyclisation alcaline

On dissout de l'acétylallylglycine (8.5 g., 0.054 mole) dans 100 ml. d'acide acétique glacial. A la température de la chambre, on ajoute goutte à goutte du brome (9 g., 0.057 mole) dissous dans 25 ml. de chloroforme en maintenant une bonne agitation. L'addition de brome terminée, on évapore le solvant sous pression réduite et on obtient un résidu gommeux qui se refuse à cristalliser. On y ajoute 150 ml. d'eau distillée et 10 ml. d'acide sulfurique concentré. Après une hydrolyse de quatre heures, on cyclise en milieu alcalin tel que déjà décrit.

La chromatographie sur papier et l'analyse des acides aminés libres montrent que ces sels sont un mélange en parties à peu près égales d'hydroxyproline et d'acide 2-amino-4,5-dihydroxyvalérianique.

On obtient le même résultat à partir de l'acide allylacétamidomalonique au lieu de l'acétylallylglycine.

Formation des sels de cuivre de l'hydroxyproline et de l'acide 2-amino-4,5-dihydroxyvalérianique

Après avoir fait l'hydrolyse acide et la cyclisation alcaline des dérivés dihalogénés ou des lactones halogénées, on précipite les ions halogénés par le carbonate d'argent. Les bromures ou chlorures d'argent sont filtrés et le filtrat

est saturé d'hydrogène sulfuré. Après filtration, on évapore la solution à sec. Le résidu est dissous dans un minimum d'eau. On prépare les sels de cuivre des acides aminés par ébullition de la solution ainsi obtenue, en présence d'un excès de carbonate cuivrique et on évapore la solution à sec sous pression réduite. On cristallise les sels de cuivre par dissolution dans un petit volume d'eau chaude et addition d'alcool éthylique. Si après quelques heures au froid rien ne précipite, on évapore un peu d'alcool.

Suivant les produits de départ utilisés, les rendements en mélange des sels de cuivre de l'acide dihydroxylé et de l'hydroxyproline varient entre 40 et 60%. Le pourcentage respectif de l'un et de l'autre des acides aminés dans ces mélanges varie suivant les produits de départ (voir les tableaux I et II).

Séparation des sels de cuivre de l'hydroxyproline et de l'acide dihydroxylé

Hydroxyprolinate de cuivre

On sèche à 110° C. un mélange de sels de cuivre (8.5 g.) des deux acides aminés obtenus suivant la méthode décrite plus haut. On reprend par un minimum d'eau à chaud et on porte au froid. Le sel de cuivre, couleur violet, de l'hydroxyproline est insoluble. On le filtre. Poids obtenu: 3.5 g. Calculé pour $C_{10}H_{16}O_6N_2Cu.\frac{1}{2}H_2O$: N, 8.42%. Trouvé: N, 8.40%.

Une partie du sel de cuivre est solubilisée dans l'eau, acidifiée par l'acide acétique, puis saturée par l'hydrogène sulfuré pour précipiter le cuivre. Après filtration, la solution est décolorée au noir animal puis évaporée à sec. On reprend le résidu à l'eau et on fait un chromatogramme. L'hydroxyproline fut identifiée par chromatographie ascendante avec le phénol comme solvant. Seule la tache jaune caractéristique de l'hydroxyproline apparaissait après révélation à la ninhydrine. R_f 0.66. La méthode employée pour isoler l'acide aminé sous forme cristalline fut celle décrite par Gaudry et Godin (5).

2-Amino-4,5-dihydroxyvalérianate de cuivre

Le filtrat, obtenu lors de la filtration de l'hydroxyprolinate de cuivre, est concentré à très faible volume. On ajoute un grand excès d'alcool éthylique et on porte au froid. Un sel de cuivre bleu très pâle précipite. Poids obtenu: 4.4 g. Par ébullition du sel de cuivre dans l'alcool méthylique absolu, on solubilise les dernières traces d'hydroxyprolinate de cuivre. Après avoir filtré, on sèche le sel de cuivre de l'acide 4,5-dihydroxyvalérianique. P. f. 200–205° C. (déc.). Calculé pour $C_{10}H_{20}O_8N_2Cu$: N, 7.78%. Trouvé: N, 7.70%.

La chromatographie révèle la présence d'une seule tache violette, soit l'acide 2-amino-4,5-dihydroxyvalérianique. R_f 0.42.

Le fractionnement des sels de cuivre par la méthode décrite plus haut donne un rendement excellent. Ainsi en partant d'un mélange de 8.5 g. on sépare 3.5 g. d'hydroxyprolinate de cuivre et 4.4 g. de 4,5-dihydroxyvalérianate de cuivre, soit un rendement total de 93%.

Chlorhydrate de la 2-amino-5-hydroxyvalérolactone

L'acide dihydroxylé sous forme libre, obtenu à partir du 2-amino-4,5-dihydroxyvalérianate de cuivre (1 g.), suivant la méthode décrite précédemment, donne une huile visqueuse qu'il est impossible de cristalliser. On reprend à l'alcool éthylique absolu et on fait barbotter de l'acide chlorhydrique gazeux

jusqu'à ce que le tout se soit solubilisé. A ce stage, on évapore à sec. Le résidu cristallin est repris par un minimum d'alcool anhydre et filtré. Poids du chlorhydrate de la lactone obtenue: 0.4 g. Rendement 43%. P. f. 203–205° C. Calculé pour $C_5H_{10}O_3NCl$: N, 8.36%; Cl, 21.1%. Trouvé: N, 8.40%; Cl, 20.7%.

Acide 2-phényluréido-4-pentènoïque

On dissout de l'allylglycine (1.8 g., 0.015 mole) dans une solution de 0.62 g. d'hydroxyde de soude dans 50 ml. d'eau distillée. A la température de la chambre, on ajoute à la solution de l'isocyanate de phényle (1.9 g., 0.015 mole) en agitant pendant 15 min. On acidifie la solution avec 1.5 ml. d'acide chlorhydrique concentré et on refroidit ensuite sur un bain de glace. On agite le précipité au froid pendant quelques minutes et on filtre le produit. Rendement: 3 g., 85.0%. P. f. 162–163° C. Calculé pour $C_{12}H_{14}O_3N_2$: N, 11.9%. Trouvé: N, 12.1%.

5-Allylhydantoïne

On dissout de l'allylglycine (8.6 g., 0.075 mole) dans 75 ml. d'eau distillée. On ajoute du cyanate de potassium (6.5 g., 0.08 mole) et on chauffe à une température de 65° C. pendant deux heures. On ajoute ensuite à la solution 17.5 ml. d'acide chlorhydrique concentré et on chauffe à 90° C. pendant deux heures. La solution est évaporée à sec sous pression réduite et le résidu est agité dans de l'acétone chaude. Le chlorure de potassium insoluble est filtré et l'acétone est évaporée à sec sous pression réduite. On reprend le résidu avec 100 ml. d'eau bouillante et on décolore la solution au noir animal. On évapore la solution à petit volume, 25 ml. environ, et on place le tout au froid. L'hydantoïne cristallise rapidement. Rendement: 7.4 g., 72%. P. f. 104° C. Calculé pour $C_6H_8O_2N_2$: N, 20.0%. Trouvé: N, 19.5%.

5-(2,3-Dichloropropyl)hydantoïne

On place de la 5-allylhydantoïne (5 g., 0.035 mole) dans 100 ml. de chloroforme. On fait barbotter du chlore gazeux dans la solution jusqu'à saturation, en maintenant cette dernière à la température de la chambre. On évapore à sec sous pression réduite en ne chauffant que légèrement. On recristallise le résidu d'un petit volume d'eau bouillante. Rendement: 3.55 g., 48.5%. P. f. 150–152° C. Calculé pour $C_6H_8O_2N_2Cl_2$: N, 13.25%; Cl, 33.3%. Trouvé: N, 13.20%; Cl, 33.2%.

5-(2,3-Dibromopropyl)hydantoïne

Dans un ballon à large col de 300 ml., on place de l'allylhydantoïne (8.55 g., 0.061 mole) dans 100 ml. de chloroforme. On ajoute goutte à goutte du brome (10.0 g., 0.062 mole) en maintenant la solution à la température de la chambre. On évapore la solution à sec sous pression réduite en ne chauffant que légèrement. On recristallise le résidu d'un volume minimum d'eau bouillante. Rendement: 17 g., 93%. P. f. 155° C. Calculé pour $C_6H_8O_2N_2Br_2$: N, 9.3%; Br, 53.0%. Trouvé: N, 9.3%; Br, 51.1%.

3-Phényl-5-allylhydantoïne

On dissout de l'allylglycine (3.5 g., 0.03 mole) dans une solution de 1.2 g. d'hydroxyde de soude dans 50 ml. d'eau distillée. On ajoute goutte à goutte

de l'isocyanate de phényle (3.62 g., 0.03 mole) en maintenant la solution à la température de la chambre. La solution est agitée pendant environ 15 min. et ensuite acidifiée avec 15 ml. d'acide chlorhydrique concentré. On chauffe la solution sur un bain-marie bouillant pendant une heure, puis on l'évapore à sec sous pression réduite. Le résidu est recristallisé de l'alcool dilué. Rendement: 4 g., 61%. P. f. 97–98° C. Calculé pour $C_{12}H_{12}O_2N_2$: N, 12.9%. Trouvé: N, 12.8%.

3-Phényl-5-(2,3-dichloropropyl)hydantoïne

A la température de la chambre, on fait barbotter du chlore gazeux dans une solution de 3-phényl-5-allylhydantoïne (2 g., 0.0092 mole) dans 100 ml. de chloroforme. Lorsque la solution est complètement saturée de chlore, on l'évapore à sec sous pression réduite. On cristallise le résidu huileux de l'alcool dilué. Rendement: 1 g., 38%. P. f. 142° C. Calculé pour $C_{12}H_{12}O_2N_2Cl_2$: N, 9.78%; Cl, 24.5%. Trouvé: N, 9.28%; Cl, 20.3%.

3-Phényl-5-(2,3-dibromopropyl)hydantoïne

Dans un ballon à large col de 300 ml., on dissout de la 3-phényl-5-allylhydantoïne (2 g., 0.0092 mole) dans 100 ml. de chloroforme. A la température de la chambre, on ajoute goutte à goutte du brome (1.5 g., 0.0094 mole). L'addition terminée, on évapore la solution à sec sous pression réduite. On recristallise le résidu de l'alcool dilué. Rendement: 2.75 g., 79%. P. f. 130–135° C. Calculé pour $C_{12}H_{12}O_2N_2Br_2$: N, 7.45%; Br, 42.5%. Trouvé: N, 7.46%; Br, 42.0%.

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THE VOLATILIZATION OF PLUTONIUM FROM NEUTRON-IRRADIATED URANIUM[1]

By D. E. McKenzie

ABSTRACT

The volatilization of plutonium from neutron-irradiated uranium has been examined at 1540°, 1650°, and 1769°C. The observed rates of evaporation of plutonium have been compared with calculated rates based on the Langmuir equation and Raoult's law. Experimental and calculated results agree within the precision of the experimental measurements, indicating that the plutonium in neutron-irradiated uranium follows the ideal solution laws.

INTRODUCTION

In some types of nuclear reactors uranium metal is used as fuel. The processing of the resulting neutron-irradiated uranium and return of the separated plutonium and uranium to the reactor may become important if such reactors are to produce economic power. Current processing methods require dissolution of the neutron-irradiated uranium and solvent extraction or ion exchange techniques to achieve the separation (1, 4). The uranium metal for re-irradiation must be prepared from the resulting aqueous solution. Alternative separation methods, in which uranium is retained as metal throughout, may offer advantages.

One possible method is the volatilization of plutonium from the uranium. This technique is suggested by the fact that, at, for example, 1540°C., the vapor pressure of plutonium (5) is 300 times that of uranium (6). This paper describes an investigation of the rate of evaporation, in vacuum, of plutonium from neutron-irradiated uranium. The results are in qualitative agreement with those obtained at the North American Aviation Laboratories in Downey, California (3).

EXPERIMENTAL

Materials and Apparatus

Neutron-irradiated uranium was obtained from rods which had been irradiated for various periods in the NRX reactor. The beryllia crucibles which were of the vertical-wall type were obtained from the Atomic Energy Research Establishment at Harwell, England. The evaporating area of a melt was calculated from the average of a number of diameter measurements of each crucible.

The apparatus for the distillation is illustrated in Fig. 1. The uranium was heated by a tungsten resistance coil, surrounded by beryllia thermal shields and molybdenum radiation shields. The assembly was mounted in a glass bulb which was evacuated by diffusion and mechanical pumps through a trap cooled by liquid air. The pumping equipment gave pressures of less than

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5×10^{-5} mm. of Hg in the system when the charge was at temperature. The system was contained in a dry box in which the glass bulb was surrounded by lead.

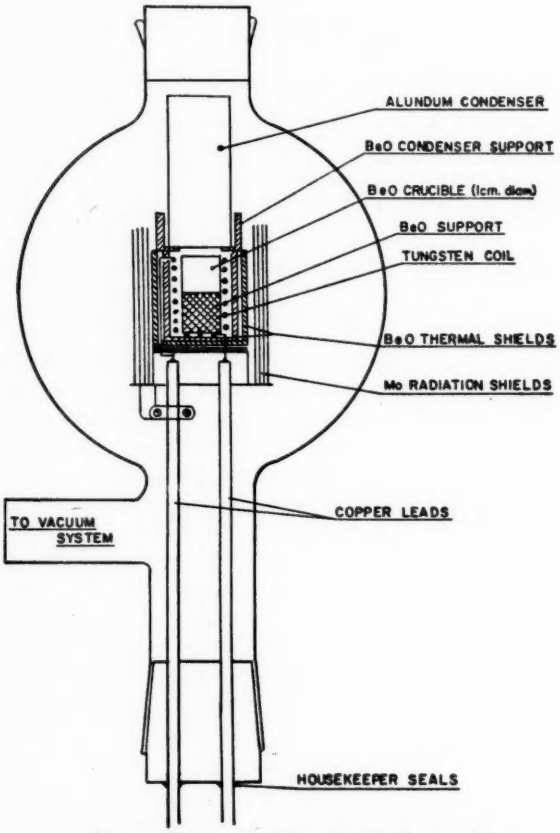

FIG. 1. Diagram of bulb and crucible assembly.

Distillations were carried out at 1540°, 1650°, and 1769°C. The temperatures 1540° and 1769°, the melting points of iron and platinum respectively, were reproduced by careful determinations of the power required to melt these metals in the crucible assembly. An interpolation of these values gave the power required for 1650°. The coils were always restandardized after two or three runs. A constant temperature during a run was maintained by observing the uranium charge with an optical pyrometer which was sighted through an optical window in the top ground-glass joint and a hole in the condenser. The maximum error in the temperature is ±15°C.

Procedure

The procedure for an experiment was as follows: A piece of neutron-irradiated uranium was etched in nitric acid to remove at least 25% of its

weight for a reference solution. The remaining uranium was washed with water and with acetone before being placed in a beryllia crucible and put in the apparatus. The bulb was pumped down to a pressure of 10^{-6} mm. of Hg and power applied to the coil to degas the crucible and associated shields. The uranium was then heated to temperature to perform the distillation. The pressure in the system did not exceed 10^{-4} mm. of Hg during the degassing operation.

After distillation the charge was cooled to room temperature and the vacuum broken. The uranium residue was removed from the crucible and dissolved in nitric acid. Also the crucible was leached in nitric acid. These solutions and the reference solution were analyzed for plutonium by standard radiochemical procedures.

RESULTS

The data for the various runs are given in Table I. The total amount of plutonium distilled was determined from the plutonium content of the original uranium and the plutonium content of the uranium residue. Only that portion

TABLE I

DATA FOR PLUTONIUM DISTILLATIONS

Run No.	Wt. U (gm.)	Time (hr.)	Area ($cm.^2$)	Pu distribution (% original)		
				Residue	Crucible	Condenser
Temperature = 1540°C.						
92	3.972	2.75	0.581	65.7	7.0	27.3
101	2.464	3.61	0.441	56.5	16.3	27.2
103	2.022	7.31	0.490	31.0	33.8	35.2
104	2.362	9.00	0.528	8.4	54.2	37.4
109	2.044	10.00	0.441	23.0	33.9	43.1
102	1.446	7.50	0.478	4.8	42.2	53.0
110	2.183	14.00	0.540	4.4	42.4	53.2
117	1.442	10.68	0.554	4.3	50.8	44.9
Temperature = 1650°C.						
81	3.460	0.82	0.581	67.1	7.7	25.2
85	4.229	2.00	0.528	38.0	25.2	36.8
82	4.017	1.75	0.581	32.6	33.6	33.8
83	4.307	3.67	0.515	25.8	35.8	38.4
84	4.027	4.27	0.586	13.4	37.4	49.2
107	1.841	3.58	0.430	3.9	40.0	56.1
106	3.393	10.37	0.407	4.3	34.7	61.0
108	3.157	7.62	0.515	1.9	40.2	57.9
116	5.487	13.58	0.502	2.0	25.5	72.5
Temperature = 1769°C.						
89	3.454	0.40	0.465	35.7	24.8	39.5
90	3.723	0.75	0.609	24.2	28.1	47.7
119	2.179	0.61	0.708	4.1	46.4	49.5
120	2.005	0.85	0.650	1.5	51.5	47.0
121	1.838	1.07	0.622	2.1	64.4	33.5

of the distilled plutonium that collected on the sides of the crucible was recovered for analysis. This plutonium fraction is due to distillation since in one run, the top of the crucible, which had been sawed directly above the uranium residue, contained essentially all the plutonium found in the crucible.

Plutonium balances were made in two runs by crushing and leaching the alundum condenser. In run 116, 94% of the original plutonium was recovered and in run 117, 85% was recovered.

DISCUSSION

The results have been correlated with theory in the following manner. W, the theoretical rate of evaporation per unit area of a pure substance in vacuum, is given by the Langmuir equation (2)

$$W = 0.0583\, p_0\sqrt{(M/T)} \qquad [1]$$

where W has the units gm. cm.$^{-2}$ sec.$^{-1}$ for the constant 0.0583,

M = the molecular or atomic weight of the vaporizing species, and

p_0 = the vapor pressure of the pure substance in mm. of Hg at the temperature T°K.

In the derivation of this equation a clean evaporating surface and uniform temperature in the melt are assumed. Although these conditions, in particular the clean surface, are not strictly adhered to, the equation will be used for the present experiments.

To apply the Langmuir equation to the evaporation of a particular component from a solution it is necessary to replace the vapor pressure of the pure substance, p_0 in equation [1], by the partial pressure of that component above the solution. For dilute metallic solutions the solute (i.e. the less concentrated component) generally obeys Henry's law which defines this partial pressure as

$$p = k\,n \qquad [2]$$

where k is a constant and n is the mole fraction of the component in solution. If the component obeys Raoult's law then k is equal to p_0. For convenience in comparing the results with Henry's and with Raoult's laws we introduce a new constant k' such that $k = k'p_0$. Thus, assuming the component follows Henry's law, the Langmuir equation for the evaporation of that component from a solution becomes

$$W = 0.0583\, k'\, p_0\, n\sqrt{(M/T)}. \qquad [3]$$

Equation [3] has been integrated, for application to the distillation of plutonium from neutron-irradiated uranium, with the following result:

$$\log Y/Y_0 = -\left(\frac{0.0583}{2.303}\, p_0\sqrt{(M/T)}\right) k'\,\frac{tA}{U_0} \qquad [4]$$

where Y/Y_0 = the fraction of the original plutonium in the residue,

t = the time of distillation in seconds,

A = the evaporating area in cm.^{2}, and

U_0 = the initial weight of the uranium in grams.

It was assumed in the integration that k' and U_0 are constants, i.e. k' is independent of plutonium concentration in the range studied, and the amount of uranium distilled is a negligible fraction of the original charge. The mole fraction of plutonium was assumed to be weight of plutonium/weight of uranium. In addition, the assumptions in applying [4] are (1) neutron-

irradiated uranium behaves as a binary solution of plutonium in uranium and (2) plutonium distills in monatomic form.

The experimental results have been correlated with equation [4] by (1) assuming Raoult's law, i.e. $k' = 1$, and calculating a theoretical value for the plutonium distilled and (2) assuming Henry's law and calculating a value for k'. The equation $\log p_{mm} = 7.895 - 17590/T$ has been used for the vapor pressure of pure plutonium (5). The correlations are summarized in Tables II, III, and IV (the units of time have been changed to hours). The experi-

TABLE II

COMPARISON OF EXPERIMENTAL AND CALCULATED DISTILLATIONS OF PLUTONIUM FOR 1540°C.
$\log Y/Y_0 = -0.516(tA/U_0)k'$

Run No.	Initial Pu conc. (wt.%)	Time × area / Wt. of U (hr. cm.² gm.⁻¹)	Pu distilled (% original)		k'
			Calc. ($k' = 1$)	Exptl.	
92	0.047	0.403	38.0	34.3	0.9
101	0.014	0.646	53.6	43.5	0.7
103	0.0068	1.77	87.8	69.0	0.6
104	0.0055	2.01	90.9	91.6	1.0
109	0.034	2.16	92.3	77.0	0.6
102	0.024	2.48	94.7	95.2	1.0
110	0.054	3.46	98.4	95.6	0.8
117	0.11	4.11	99.2	95.7	0.6

TABLE III

COMPARISON OF EXPERIMENTAL AND CALCULATED DISTILLATIONS OF PLUTONIUM FOR 1650°C.
$\log Y/Y_0 = -1.797(tA/U_0)k'$

Run No.	Initial Pu conc. (wt.%)	Time × area / Wt. of U (hr. cm.² gm.⁻¹)	Pu distilled (% original)		k'
			Calc. ($k' = 1$)	Exptl.	
81	0.031	0.138	43.5	32.9	0.7
85	0.086	0.250	64.4	62.0	0.9
82	0.082	0.253	64.9	67.4	1.1
83	0.052	0.439	83.7	74.2	0.7
84	0.066	0.622	92.4	86.6	0.8
107	0.054	0.836	96.8	96.1	1.0
106	0.0038	1.24	99.4	95.7	0.6
108	0.024	1.24	99.4	98.1	0.8
116	0.020	1.24	99.4	98.0	0.8

TABLE IV

COMPARISON OF EXPERIMENTAL AND CALCULATED DISTILLATIONS OF PLUTONIUM FOR 1769°C.
$\log Y/Y_0 = -5.950(tA/U_0)k'$

Run No.	Initial Pu conc. (wt.%)	Time × area / Wt. of U (hr. cm.² gm.⁻¹)	Pu distilled (% original)		k'
			Calc. ($k' = 1$)	Exptl.	
89	0.067	0.054	52.8	64.3	1.4
90	0.085	0.123	81.4	75.8	0.8
119	0.24	0.198	93.4	95.9	1.2
120	0.13	0.275	97.7	98.5	1.1
121	0.011	0.361	99.3	97.9	0.8

mental results are compared with calculations based on Raoult's law in Figs. 2, 3, and 4 where log Y/Y_0 is plotted against tA/U_0. The effect of an uncertainty in temperature of ± 15°C. is shown in these figures.

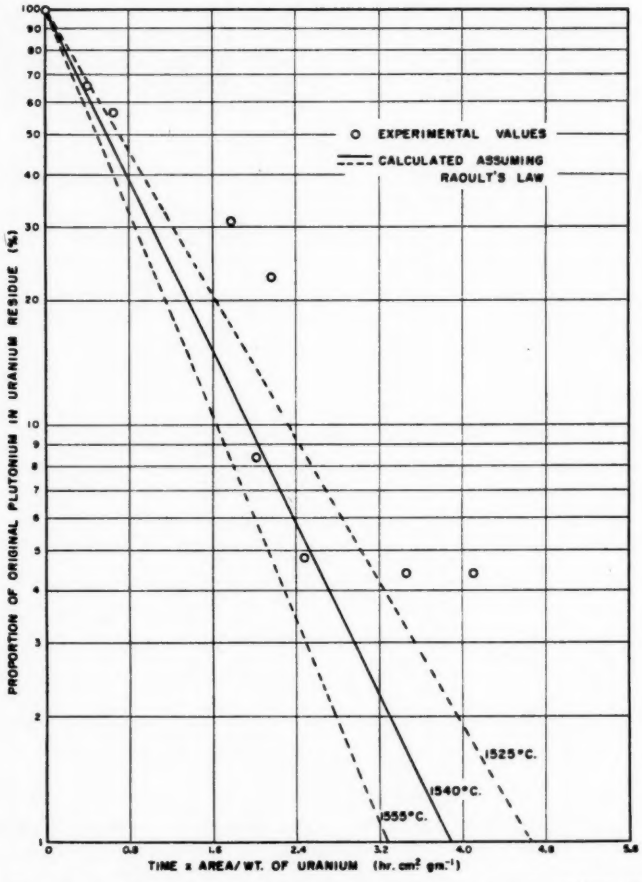

FIG. 2. Comparison of calculated and experimental results for runs at 1540°C. The dotted lines indicate the effect, on the calculations, of an error of ± 15°C. in temperature.

The quantity k' is the activity coefficient of plutonium in neutron-irradiated uranium. Its variation with temperature can be related to the heat of solution of plutonium in this material. However, the estimated error in the values of k' obtained by the present procedures is about $\pm 25\%$ (the uncertainty in temperature alone results in an error of $\pm 17\%$). The average value of k' for each temperature deviates from unity by less than this experimental error.

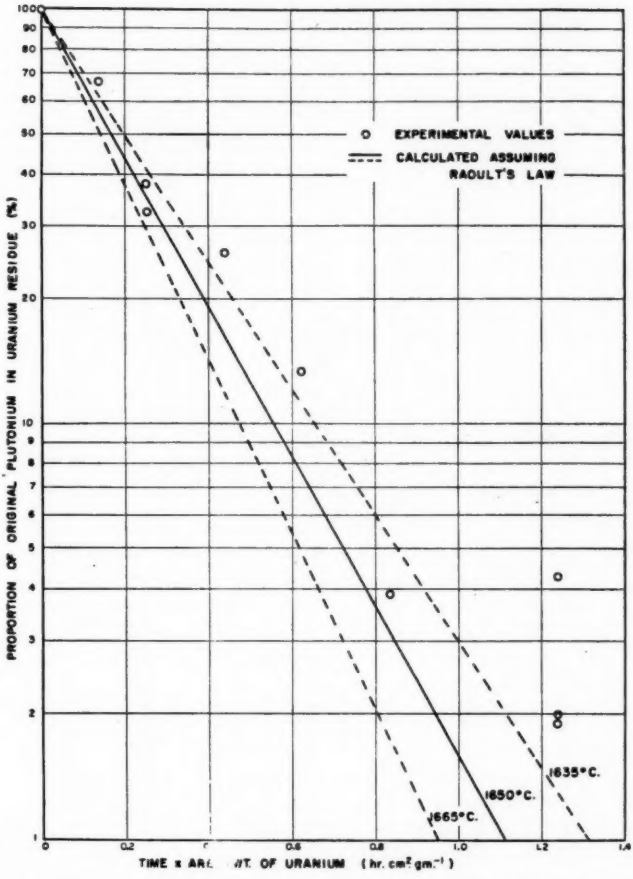

FIG. 3. Comparison of calculated and experimental results for runs at 1650°C.

In addition, no effect due to varying plutonium concentrations is observed. Thus, for these temperatures and concentrations, it is concluded that plutonium in neutron-irradiated uranium forms essentially an ideal solution.

This conclusion might have been predicted from the proximity of plutonium to uranium in the actinide series. Deviations of k' from unity, if real, are probably associated with assumptions made in the treatment of the data. Uranium, since it is an excellent "getter" for oxygen, would not be expected to have a clean evaporating surface. Also plutonium may form high melting point compounds with one or more of the fission products. Both of these effects would lower the evaporation rate from that predicted from the Langmuir equation and Raoult's law.

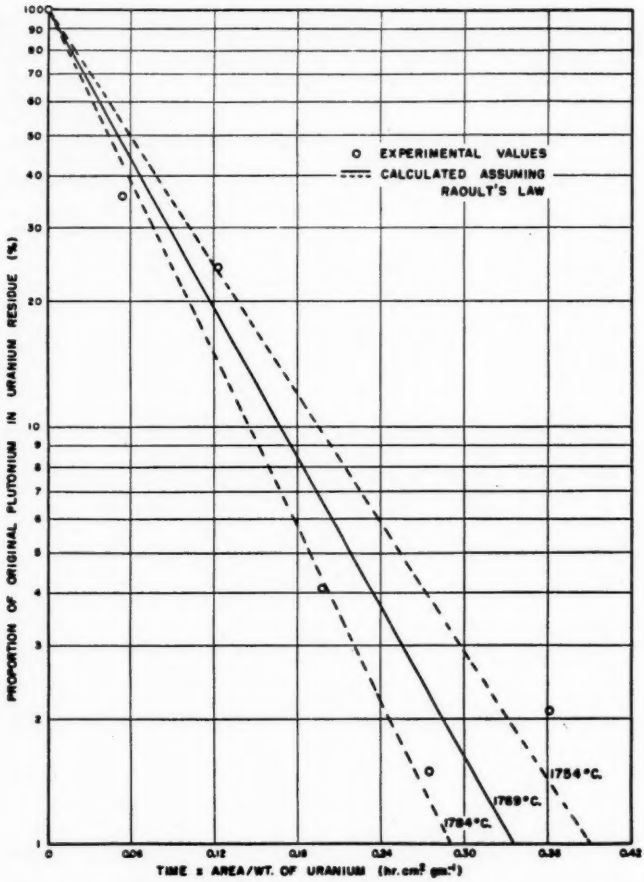

FIG. 4. Comparison of calculated and experimental results for runs at 1769°C.

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THE ELUCIDATION OF THE STRUCTURE OF HYOCHOLIC ACID[1]

By P. Ziegler

ABSTRACT

Hyocholic acid, a new bile acid isolated from hog bile, was identified as 3(α), 6(α),7(α)-trihydroxycholanic acid.

The bile acids are the main constituents of animal bile where they are usually found in conjugation with amino acids. In the past it has been the practice to saponify bile under rigorous alkaline conditions prior to isolation of the constituent bile acids and, in this manner, the compositions of the bile acid mixtures from a large number of animals were determined. Hog bile proved to be an exception despite a considerable amount of effort, and the greater portion of the saponified hog bile solids could never be accounted for in the form of identifiable bile acids or their derivatives (7, 8). This has prompted a careful investigation of hog bile in these laboratories resulting in the isolation of a hitherto unknown acid which, according to established terminology, will be named hyocholic acid.

By the procedure of isolation (to be reported in more detail at a later date) hyocholic acid was obtained as the methylated and acetylated derivative which gave satisfactory analyses for I and also was found, by acetate determination, to contain three moles of acetate per mole of steroid. This compound was transparent to ultraviolet from 220 to 400 mμ, failed to react with tetranitromethane or ketone reagents, and, on infrared examination, exhibited the characteristic bands of the carbomethoxy and acetate groups. Saponification of I afforded hyocholic acid (II) which analyzed correctly for a trihydroxycholanic acid; it was not identical with the widely distributed cholic acid and a literature survey failed to reveal any bile acid with properties similar to II. It was later found that the methyl ester of hyocholic acid gave a positive Malaprade test, indicating that at least two of the three hydroxyl groups were adjacent to each other, an arrangement unknown in the natural bile acids.

Hyocholic acid (II) was then oxidized with chromic acid to give, after methylation, 3-ketothilobilianic acid trimethyl ester (III), not previously described. This compound gave a positive Zimmermann reaction and analyzed correctly for three methoxyl groups. Obviously the carbon–carbon bond of the vicinal glycol was ruptured giving rise to two new carboxyl groups, while the third hydroxyl function was oxidized to a ketone. This proved that all three hydroxyl groups were secondary alcohols and that one of these was isolated from the glycol structure and not adjacent to it. The positive Zimmermann reaction showed that the ketone was not located at positions 6, 7, or 12 of the nucleus. It now remained to determine which of the rings had broken during the oxidation; this would locate at once the positions of the two vicinal

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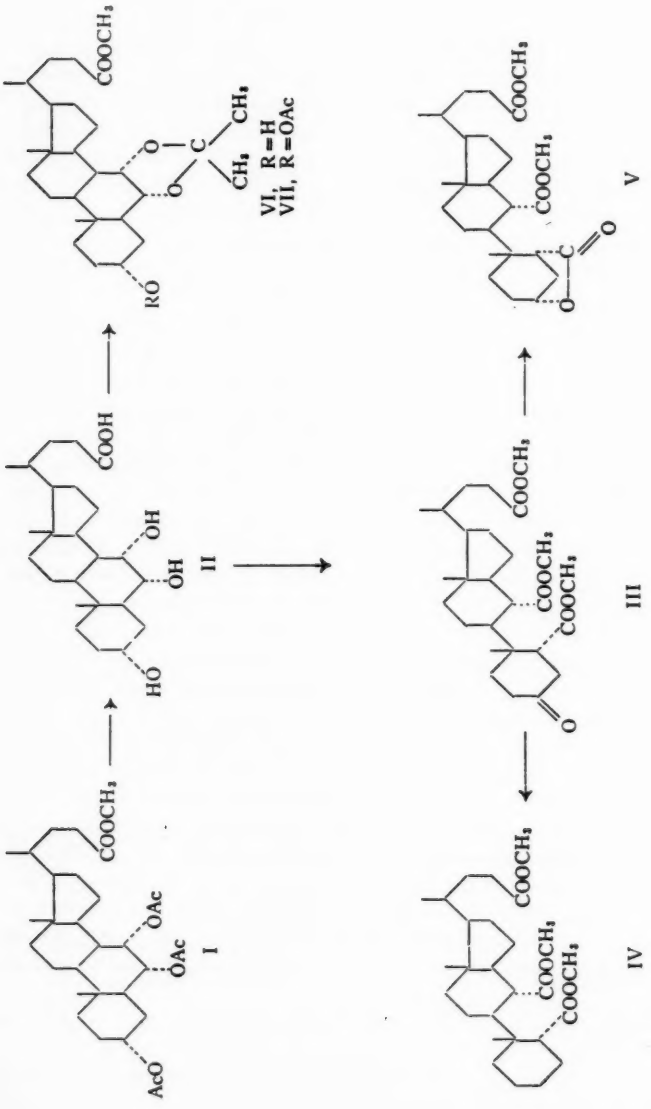
Contribution from the Research and Development Laboratories of Canada Packers Ltd., Toronto, Ont.

hydroxyl groups. It was then assumed that the isolated hydroxyl group in hyocholic acid (II) was present at C-3, because all naturally occurring bile acids have this characteristic grouping and because the positive Zimmermann test exhibited by III lent further evidence for that, although this did not



constitute a rigid proof. That assumption at once eliminated ring A as the site of the glycol which previously was shown to be removed by at least two carbon atoms from the third hydroxyl group. Ring C also could be excluded because all possible isomers of 3(α),11,12-trihydroxycholanic acid are synthetic products described in the literature (2, 11) and none of these compounds had the physical properties of hyocholic acid. That left rings B and D as the only possible locations for the vicinal hydroxy groups. Ring D seemed an unlikely prospect since hydroxylation in that ring is not known to occur in bile acids. Clearly then ring B was suspected and circumstantial evidence could be derived from the fact that hog bile contains 20% of 3(α),6(α)-dihydroxycholanic acid and an appreciable quantity of 3(α),7(α)-dihydroxycholanic acid. These acids (hyo- and cheno-desoxycholic acid respectively) are produced in the liver from cholesterol and it seemed reasonable to think that the enzymes responsible for hydroxylation at C-6 and C-7 could also produce compounds that are doubly hydroxylated, giving rise to the 6,7-vicinal glycol.

Definite proof for this was then obtained from two products derived from 3-ketothilobilianic acid trimethyl ester (III). This compound was reduced by Huang-Minlon's modification of the Wolff–Kishner reduction (4) to give, after methylation, IV which was found to be identical with the thilobilianic acid trimethyl ester prepared by Wieland (10). Sodium borohydride reduction of III and subsequent esterification gave V which was found to contain only two methoxyl groups. Infrared examinations showed conclusively the presence of a five-membered lactone ring by the very characteristic absorption bands at 940 and 1775 $cm.^{-1}$ (3, 5). This lactone (V) has also been prepared by Japanese workers (13) by nitric acid oxidation of 3(α)-acetoxy-6(or 7)-ketocholanic acid.

The above experiments furnished proof for the location of the hydroxyl groups at positions 3, 6, and 7. It now remained to determine the stereochemical configuration of these groups. It was found that hyocholic acid did not give an insoluble digitonide, which indicated that the hydroxyl group at C-3 was in the α-position. Conclusive evidence for this was also obtained from V which was identical with the lactone obtained by the Japanese investigators (13) whose starting material contained the 3(α)-hydroxy group; during their sequence of reactions no change took place involving in any way the stereochemistry of the alcohol group. In our hands, V was obtained by sodium borohydride reduction of the ketone III and it is well known (6) that such reductions yield predominantly 3(α)-hydroxy compounds in the A/B *cis* series and 3(β)-hydroxy analogues in the A/B *trans* series. Furthermore, the presence of the lactone in V is also excellent proof not only for the α-configuration of the 3-hydroxy group but also for the A/B *cis* junction. Five-membered lactones are planar and as such require that their two bonds, which are the links to ring A, be in the same plane, either both α or both β. There is however a difference between the two as regards ease of formation. Lactones of the α-type such as V form spontaneously from the corresponding hydroxy acids which cannot be isolated (3, 13); on the other hand, 3(β)-hydroxy-5(β)-carbohydroxy steroids exist as such and lactonize only after

treatment with hot acetic anhydride or under similar conditions (12). In our work the hydroxy compound was not isolated but only the lactone which must have formed during acidification in the cold of the alkaline sodium borohydride reaction mixture. Clearly this represents ample support for the α-configuration of the lactone in V.

The hydroxyl groups at C-6 and C-7 can exist in four different stereochemical positions in two of which they are in the same plane (6α,7α or 6β,7β). It is known that only those vicinal glycols in which the alcoholic functions are in the same plane form acetonides (9). The methyl ester of hyocholic acid (II) was therefore treated with acetone and *p*-toluenesulphonic acid, but no crystalline substance could be obtained. The residue was then acetylated and the products chromatographed on alumina. Nearly all the material was eluted with benzene but again no crystals could be isolated. The benzene fraction was found to contain only one acetate group per mole of steroid. Another portion of the benzene eluate was then examined in the infrared and no trace of a free hydroxyl group could be detected. Therefore the acetonides VI and VII must have been formed and the hydroxyl groups in II are in the same plane. Additional support for acetonide formation was also obtained by refluxing VII with acetic acid which decomposed the acetonide to give acetone. After dilution with water, the mixture was distilled and the distillate gave a positive iodoform test.

The above experiments then left only two possibilities for the structure of hyocholic acid, the one having the glycol at 6(α),7(α), the other at 6(β),7(β). A differentiation between these two can be made on the basis of molecular rotation data which have recently been very useful in structure elucidation of steroids. The fundamental concept consists of the usually valid claim that a given functional group at a given location and configuration will always contribute the same amount to the molecular rotation of the whole molecule; or, in other words, the molecular rotation of a compound is the algebraic sum of the rotatory contributions of the constituent parts of the molecule. This holds true only if there are no "vicinal effects" which result from the interaction of functional groups which are situated close to each other in space.

From the data of Barton and Klyne (1) and from our own work, we have determined the individual rotatory contributions (all values in alcohol) of hydroxyl groups in ring B of the normal bile acid series. Lithocholic acid was found to have $[M]_D$ +121 and from these values the approximate molecular rotations of the two alternative structures for hyocholic acid should be as given below:

$[M]_D$ of 3(α),6(α),7(α)-trihydroxycholanic acid = +121−62−72 = −13,

$[M]_D$ of 3(α),6(β),7(β)-trihydroxycholanic acid = +121+24+103 = +248.

Hyocholic acid was found to have $[M]_D$ +19, which agrees more closely with the 6(α),7(α)-structure, though the approximation is not nearly as satisfactory as would be expected if there were no "vicinal effects". But it must be realized immediately that such "vicinal effects" are operative in hyocholic acid because of the interaction within the glycol grouping. An examination of the above data reveals that both the 6(α)- and the 7(α)-hydroxy contributions are

negative, while the opposite configurations give positive increments. It is reasonable to predict that, where "vicinal effects" exist, the numerical value of a rotatory contribution will be smaller than the calculated "non-vicinal" value, provided the latter has a numerical value which is not close to zero. If this is applied to the 6(α),7(α)-structure, the $[M]_D$ value would come close to +19 which was determined experimentally for hyocholic acid. At the same time the vicinal contributions of the two hydroxyl groups, though numerically reduced, would not change in sign (negative). On the other hand, in the 6(β), 7(β)-structure, the combined "non-vicinal" contributions are +127 which would have to change to −102 to accommodate the experimentally determined value of $[M]_D$ +19. This is extremely unlikely.

Besides the above evidence obtained from rotation data, there is also available some biogenetic indication for the α-configuration of the hydroxyl groups in ring B. An examination of the known constituents of hog bile shows that some compounds have hydroxyl groups at 6(α) or 7(α), but not a single hog bile acid has a hydroxyl group in the β-configuration in ring B.

From the data presented it was concluded that hyocholic acid is 3(α),6(α), 7(α)-trihydroxycholanic acid.

EXPERIMENTAL[2,3,4]

The Isolation of Methyl Hyocholate Triacetate (I)

Hog bile (1 liter) was refluxed with sodium hydroxide (100 gm.) for 60 hr. The solution was then acidified, extracted with ethyl acetate, and the solvent extract evaporated to a small volume. After cooling, the resulting precipitate was filtered off to yield 55 gm. of material, m.p. 150–160°C. This was treated with ethereal diazomethane and the crude methyl esters were crystallized from benzene to provide methyl hyodesoxycholate – benzene complex (23 gm.). The filtrate was evaporated *in vacuo* and the dry residue was then refluxed with pyridine (50 ml.) and acetic anhydride (250 ml.) for four hours. Evaporation of the solvents, extraction with ether, washing, and crystallization from hexane or iso-octane afforded crude I (15 gm.), m.p. 173–176°C. This was recrystallized several times from ether–hexane to give transparent, irregular plates, m.p. 188–190°C. and $[\alpha]_D^{20}$ +20.95° (*c*, 1.22, dioxane). Infrared absorption bands at 1040, 1165, 1225–1250, 1362, 1735–1740, and 2950 $cm.^{-1}$. This compound was found to contain 33.1% of acetate (calculated value = 32.27%). Analysis: Calcd. for $C_{31}H_{48}O_8$: C, 67.85; H, 8.82; O, 23.33. Found: C, 67.85, 67.81; H, 8.90, 8.88; O, 23.24, 23.34.

Hyocholic Acid (II)

Refluxing I with 4% methanolic potassium hydroxide for two hours provided, after several recrystallizations from ethyl acetate, prismatic needles of II, m.p. 183–185°C. and $[\alpha]_D^{24}$ +4.59° (*c*, 1.18, dioxane) and $[\alpha]_D^{25}$ +4.59° (*c*, 1.10, ethanol). Analysis: Calcd. for $C_{24}H_{40}O_5$: C, 70.55; H, 9.87; O, 19.58. Found: C, 70.78, 70.79; H, 9.75, 9.84; O, 19.64, 19.48.

[2] *The microanalyses were kindly performed by Mr. E. Thommen, Basel, Switzerland; the acetate determinations by Mr. C. K. Cross in our laboratories.*

[3] *The infrared examinations were made through the courtesy of Dr. G. D. Laubach of Ch. Pfizer & Co., Brooklyn, N.Y., and Miss Kirby of the Ontario Research Foundation, Toronto.*

[4] *The procedure for the isolation of I was developed in collaboration with Mrs. G. C. Buckley and Mr. A. A. Amos.*

3-Ketothilobilianic Acid Trimethyl Ester (III) from II

Compound II (2 gm.) was dissolved in acetic acid (25 ml.) and there was then added over a period of one hour a solution of chromic acid (1.8 gm.) in water (1 ml.) and acetic acid (25 ml.). The temperature was kept at 15–20°C. during the addition and for 14 hr. thereafter. The excess oxidizing agent was destroyed by sodium bisulphite and the solvents were then removed *in vacuo* at 30°C. The residue was taken up in dilute sulphuric acid and ether, the aqueous phase was extracted two more times with ether, the combined ether extracts were washed with dilute acid, then with water, and finally dried and evaporated to dryness. After methylation with ethereal diazomethane, the residue was crystallized from ether–hexane to afford 854 mgm. of material, m.p. 130–137°C. Several recrystallizations from ether–hexane yielded prismatic needles, m.p. 140–142°C. and $[\alpha]_D^{25}$ −5.05° (*c*, 0.29, dioxane). This compound (III) gave a positive Zimmermann reaction, failed to give a coloration with alcoholic ferric chloride, tetranitromethane, or triphenyltetrazolium, and did not absorb in the ultraviolet region. Infrared examination revealed strong bands at 1165, 1220, 1445, 1730, and 2930 cm.$^{-1}$ and several weaker bands in the region of 1100 to 1400 cm.$^{-1}$. Analysis: Calcd. for $C_{27}H_{42}O_7$: C, 67.75; H, 8.85; OCH_3, 19.45. Found: C, 67.81, 67.93; H, 8.82, 8.93; OCH_3, 19.33, 19.35.

Thilobilianic Acid Trimethyl Ester (IV) from III

Compound III (250 mgm.) was dissolved in diethylene glycol (8 ml.), then a hot solution of potassium hydroxide (2 gm.) in the same solvent (12 ml.) was added. After addition of 85% hydrazine hydrate (3 ml.), the solution was refluxed for one hour, the condenser was then set downwards, and the solution distilled until the internal temperature had reached 200°C. The solution was then refluxed for two hours, cooled, diluted with water, acidified with hydrochloric acid, and extracted three times with ether. The solvent extracts were washed first with dilute acid, then with water, and subsequently dried over sodium sulphate and evaporated to dryness to yield an amorphous residue (162 mgm.). This was methylated with diazomethane, then crystallized from aqueous methanol to give 82 mgm. of impure IV, m.p. 95–100°C. Two recrystallizations from iso-octane furnished IV, m.p. 108–109°C. and $[\alpha]_D^{24}$ −13.6° (*c*, 0.695, dioxane). Wieland (10) cites m.p. 109°C. Analysis: Calcd. for $C_{27}H_{44}O_6$: C, 69.79; H, 9.55; O, 20.66; OCH_3, 20.03. Found: C, 69.92; H, 9.36; O, 21.39; OCH_3, 19.84.

3(α)-Hydroxythilobilianic Acid – 3,5-Lactone Dimethyl Ester (V) from III

Compound III (100 mgm.) was dissolved in methanol (15 ml.) and the solution was cooled to 5°C. There was then added, in five portions, sodium borohydride (60 mgm.). The solution was kept at 20°C. overnight; it was then diluted and acidified and the products extracted with ether several times. Working up in the usual way, methylating, and crystallizing from ether–hexane afforded 47 mgm. of substance, m.p. 152–153°C. This was dissolved in ether–hexane, the solution heated to remove the ether and cooled; in this manner, irregular, transparent plates, m.p. 157–159°C. and $[\alpha]_D^{24}$ −18.9° (*c*, 0.61, dioxane), were obtained. Infrared examination showed the typical

five-membered lactone absorption at 940 and 1775 cm.$^{-1}$ as well as bands at 1725 and 1750 cm.$^{-1}$. This compound did not give a color reaction in the Zimmermann test. Analysis: Calcd. for $C_{26}H_{40}O_6$: C, 69.61; H, 8.99; O, 21.40; OCH_3, 13.84. Found: C, 69.82; H, 9.08; O, 21.57; OCH_3, 13.52.

Acetonide Formation (VI and VII)

Hyocholic acid (250 mgm.) was methylated with diazomethane and the excess reagent was removed *in vacuo*. After thorough drying, the residue was dissolved in dry acetone (20 ml.), *p*-toluenesulphonic acid (50 mgm.) was added, and the stoppered flask kept at 20–25°C. for 20 hr. The solution was neutralized with sodium bicarbonate, diluted with water, and exhaustively extracted with ether. The solvent extracts were washed, dried, and evaporated to dryness to give a residue (284 mgm.) which could not be induced to crystallize, despite chromatography on alumina.

This residue (VI) was then acetylated at 20°C. with pyridine (3 ml.) and acetic anhydride (5 ml.) for 17 hr. The solvents were removed *in vacuo* at 30°C. and again the residue did not crystallize. It was dissolved in benzene (25 ml.), poured over a column of alumina (10 gm.) and the adsorbent washed with benzene (100 ml.). The combined benzene eluates were evaporated to dryness to give the residue (223 mgm.) which was used for the subsequent tests.

(*a*) Infrared analysis showed that the material did not contain any free hydroxyl groups. The main bands were at 880, 1040, 1050, 1260, 1370, 1385, 1465, 1740, and 2875 cm.$^{-1}$.

(*b*) 45.8 mgm. of the residue required 4.03 ml. of 0.02438 *N* sodium hydroxide in the acetate determination; this indicates the presence of 12.7% acetate in VII. The calculated value (for one mole of acetate) is 11.7%.

(*c*) *Iodoform test.*—Approximately 100 mgm. of amorphous VII was dissolved in acetic acid (10 ml.) and the solution refluxed for one-half hour. The solution was diluted with water (40 ml.) and the resulting mixture distilled until 10 ml. of distillate was collected. The distillate was made strongly alkaline by addition of 10% sodium hydroxide and then the potassium iodide – iodine reagent was added. An immediate precipitation of yellow iodoform resulted. A blank did not produce any precipitate.

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SYNTHESES AND ABSORPTION SPECTRA OF 1-CHLOROPHENYL-3-PHENYL-4-ALKYL-5-PYRAZOLONES AND PYRAZOLONES-4-C^{14} [1]

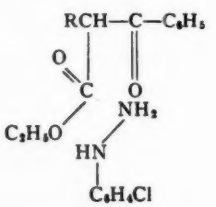
By Paul E. Gagnon, Jean L. Boivin,[2] and Yvon Laflamme[3]

ABSTRACT

Monosubstituted benzoylacetic esters $C_6H_5COCH(R)CO_2C_2H_5$ (R = H, C_nH_{2n+1} (n = 1 to 9), and $C_6H_5CH_2$) obtained by the condensation of n-alkyl halides with ethyl benzoylacetate were reacted with o-, m-, and p-chlorophenylhydrazines to give 1-chlorophenyl-3-phenyl-4-alkyl-5-pyrazolones. Pyrazolones-4-C^{14} were prepared by the action of the same hydrazines on ethyl benzoylacetate-α-C^{14} obtained from benzoyl chloride and ethyl malonate-2-C^{14}. Their activities were 801, 894, and 847 c./min. respectively. The ultraviolet and infrared absorption spectra of all the pyrazolones were determined and the most probable structures ascribed to the compounds.

INTRODUCTION

The condensation of hydrazine and its monosubstituted derivatives with β-keto esters (I) gives by elimination of water a hydrazone (II) which, by elimination of another molecule of water, produces an alkoxypyrazole (III) or a pyrazolone (IV) by elimination of alcohol.



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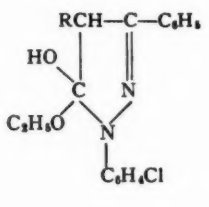

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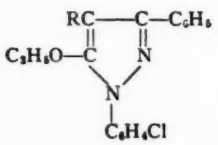

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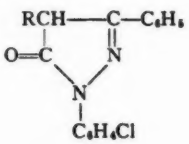

IV

The main product is an ethoxypyrazole when the reagents are heated in acid media and a pyrazolone when they are heated alone.

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Contribution from the Department of Chemistry, Laval University, Quebec, Que. This paper constitutes part of a thesis submitted to the Graduate School, Laval University, in partial fulfillment of the requirements for the degree of Doctor of Science.
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TABLE I

1-*o*-CHLOROPHENYL-3-PHENYL-4-MONOALKYL-5-PYRAZOLONES $RC_{16}H_{10}ON_2Cl$

R	M.p.,°C.	Yield, %	Formula	Analysis						Ultraviolet absorption maxima	
				Calculated, %			Found, %				
				C	H	N	C	H	N	Å	log E_m
H	170–171	86	$C_{15}H_{11}ON_2Cl$	66.54	4.09	10.34	66.35	4.18	10.47	3200 2540	3.798 4.292
CH_3	171–172	70	$C_{16}H_{13}ON_2Cl$	67.48	4.60	9.83	67.33	4.52	9.76	3050 2620	3.896 4.341
C_2H_5	121–122	67	$C_{17}H_{15}ON_2Cl$	68.33	5.06	9.37	68.27	5.07	9.36	3100 2580	3.633 4.376
C_3H_7	137–138	53	$C_{18}H_{17}ON_2Cl$	69.26	5.47	8.95	69.13	5.45	9.16	3100 2600	3.473 4.343
C_4H_9	130–131	74	$C_{19}H_{19}ON_2Cl$	69.82	5.85	8.57	69.80	5.90	8.81	3100 2600	3.718 4.349
C_5H_{11}	120–121	55	$C_{20}H_{21}ON_2Cl$	70.47	6.21	8.21	70.65	6.07	8.17	3100 2480	3.729 4.208
C_6H_{13}	122–123	51	$C_{21}H_{23}ON_2Cl$	71.07	6.53	7.89	70.94	6.47	8.07	3000 2600	3.873 4.172
C_7H_{15}	109–110	45	$C_{22}H_{25}ON_2Cl$	71.62	6.80	7.59	71.88	6.72	7.80	3050 2560	3.808 4.232
C_8H_{17}	103–104	56	$C_{23}H_{27}ON_2Cl$	72.13	7.10	7.31	72.18	7.04	7.60	2980 2600	3.751 4.246
C_9H_{19}	103–104	45	$C_{24}H_{29}ON_2Cl$	72.21	7.36	7.05	72.38	7.29	7.19	3100 2560	3.760 4.168
$C_6H_5CH_2$	157–158	78	$C_{22}H_{17}ON_2Cl$	73.22	4.75	7.76	73.17	4.71	7.64	3000 2620	3.398 4.283

TABLE II

1-*m*-CHLOROPHENYL-3-PHENYL-4-MONOALKYL-5-PYRAZOLONES $RC_{16}H_{10}ON_2Cl$

R	M.p.,°C.	Yield, %	Formula	Analysis						Ultraviolet absorption maxima	
				Calculated, %			Found, %				
				C	H	N	C	H	N	Å	log E_m
H	108–109	81	$C_{15}H_{11}ON_2Cl$	66.54	4.09	10.34	66.56	4.13	10.32	3000 2600	3.970 4.394
CH_3	160–161	57	$C_{16}H_{13}ON_2Cl$	67.48	4.60	9.83	67.51	4.49	9.85	3050 2560	3.867 4.017
C_2H_5	105–106	64	$C_{17}H_{15}ON_2Cl$	68.33	5.06	9.37	68.17	5.06	9.39	3100 2520	3.438 4.394
C_3H_7	109–110	57	$C_{18}H_{17}ON_2Cl$	69.26	5.47	8.95	69.37	5.54	9.17	3000 2540	3.862 4.268
C_4H_9	119–120	69	$C_{19}H_{19}ON_2Cl$	69.82	5.85	8.57	69.97	6.05	8.61	2980 2540	3.403 4.119
C_5H_{11}	120–121	57	$C_{20}H_{21}ON_2Cl$	70.47	6.21	8.21	70.40	6.14	8.33	2980 2480	3.804 4.222
C_6H_{13}	140–141	49	$C_{21}H_{23}ON_2Cl$	71.07	6.53	7.89	71.05	6.57	8.10	3150 2500	3.559 4.366
C_7H_{15}	102–103	46	$C_{22}H_{25}ON_2Cl$	71.62	6.80	7.59	71.73	6.87	7.68	3100 2520	3.585 4.328
C_8H_{17}	111–112	52	$C_{23}H_{27}ON_2Cl$	72.13	7.10	7.31	71.85	7.03	7.45	2980 2480	3.457 4.322
C_9H_{19}	118–119	49	$C_{24}H_{29}ON_2Cl$	72.61	7.36	7.05	72.65	7.54	7.15	3100 2600	3.326 4.134
$C_6H_5CH_2$	161–162	62	$C_{22}H_{17}ON_2Cl$	73.22	4.75	7.76	73.31	4.71	7.81	2980 2520	3.642 4.108

TABLE III

1-*p*-CHLOROPHENYL-3-PHENYL-4-MONOALKYL-5-PYRAZOLONES $RC_{16}H_{10}ON_2Cl$

R	M.p.,°C.	Yield, %	Formula	Analysis						Ultraviolet absorption maxima	
				Calculated, %			Found, %				
				C	H	N	C	H	N	Å	log E_m
H	161–162	64	$C_{16}H_{11}ON_2Cl$	66.54	4.09	10.34	66.56	4.23	10.56	3050 2520	3.723 4.197
CH_3	160–161	70	$C_{16}H_{13}ON_2Cl$	67.48	4.60	9.83	67.20	4.64	9.93	3150 2540	3.854 4.280
C_2H_5	117–118	67	$C_{17}H_{15}ON_2Cl$	68.33	5.06	9.37	68.15	5.18	9.50	3000 2480	3.842 4.131
C_3H_7	182–183	65	$C_{18}H_{17}ON_2Cl$	69.26	5.47	8.95	69.04	5.34	9.03	3000 2500	3.395 4.177
C_4H_9	125–126	49	$C_{19}H_{19}ON_2Cl$	69.82	5.85	8.57	69.70	5.74	8.77	3050 2560	3.778 4.220
C_5H_{11}	167–168	56	$C_{20}H_{21}ON_2Cl$	70.47	6.21	8.21	70.48	5.93	8.45	3150 2600	3.738 4.257
C_6H_{13}	152–153	45	$C_{21}H_{23}ON_2Cl$	71.07	6.53	7.89	71.32	6.44	8.12	3100 2520	3.740 4.249
C_7H_{15}	112–113	42	$C_{22}H_{25}ON_2Cl$	71.62	6.80	7.59	71.65	6.84	7.62	3150 2500	3.760 4.312
C_8H_{17}	117–118	49	$C_{23}H_{27}ON_2Cl$	72.13	7.10	7.31	72.11	7.15	7.34	3050 2580	3.454 4.297
C_9H_{19}	113–114	39	$C_{24}H_{29}ON_2Cl$	72.61	7.36	7.05	72.48	7.19	7.28	2960 2520	3.686 4.237
$C_6H_5CH_2$	165–166	50	$C_{23}H_{17}ON_2Cl$	73.22	4.75	7.76	73.25	4.88	7.89	2980 2480	3.469 4.332

One of the objects of the present investigation was to synthesize and to determine the structure of 1-chlorophenyl-3-phenyl-4-alkyl-5-pyrazolones. Another object was to prepare pyrazolones-4-C^{14}.

The starting esters used in the present work, the ethyl-α-monosubstituted-α-benzoylacetates, were prepared (9) by alkylation of ethyl benzoylacetate. The *o*-, *m*-, and *p*-chlorophenylhydrazines were prepared by diazotation of their respective anilines followed by reduction with sodium sulphite. The ethyl benzoylacetate-α-C^{14} was obtained by the condensation of benzoyl chloride with ethyl malonate-2-C^{14} and decarboxylation (1, 8).

The individual properties and analyses of all pyrazolones prepared are listed in Tables I, II, and III.

Ultraviolet Absorption Spectra

The ultraviolet absorption spectra of all pyrazolones were determined in alcohol solution. The results obtained are given in Tables I, II, and III and shown graphically in Figs. 1, 2, and 3.

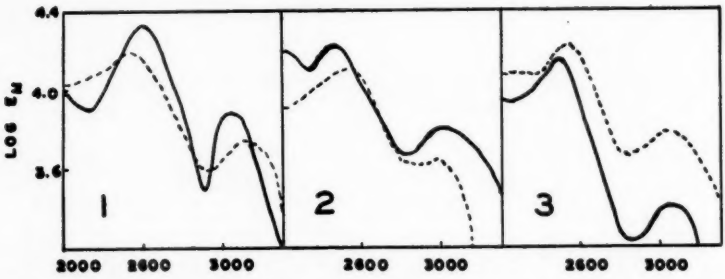
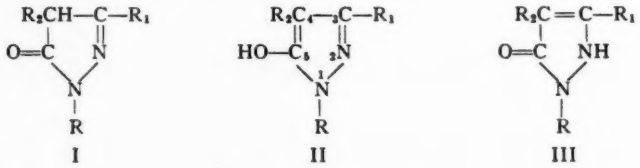


FIG. 1. Ultraviolet absorption spectra of 1-*o*-chlorophenyl-3-phenyl-4-alkyl-5-pyrazolones; ——— 4-methyl, --- 4-amyl.
FIG. 2. Ultraviolet absorption spectra of 1-*m*-chlorophenyl-3-phenyl-4-alkyl-5-pyrazolones; ——— 4-amyl, ---- 4-benzyl.
FIG. 3. Ultraviolet absorption spectra of 1-*p*-chlorophenyl-3-phenyl-4-alkyl-5-pyrazolones; ——— 4-propyl, ---- 4-butyl.

The pyrazolones have a tendency towards tautomerism and three structures may be postulated for the pyrazolones prepared.



The 4,4-dialkyl-pyrazolones as well as the pyrazolone dyes are derived from formula I. Formula II is invoked to explain the formation of alkoxypyrazoles and the formation of sodium salt. Formula III explains the easy methylation of the nitrogen atom 2.

The spectra exhibited by many pyrazolones have been studied intensively by many workers (2, 3, 4, 5, 6). These authors related the wavelength of the

absorption band and its intensity to the position of the double bond in the ring. It was found that a high intensity maximum at short wavelength was indicative of a double bond between two carbon atoms while a low intensity maximum at longer wavelength corresponded to a double bond between the nitrogen atom 2 and the carbon atom 3. The presence of two maxima for the same compound was attributed to the presence of two tautomeric forms (7).

The spectra of the *o*-, *m*-, and *p*-chlorophenylpyrazolones prepared are quite similar in their characteristics. In neutral solution these pyrazolones exhibit two maxima, one of high intensity at short wavelength and one of low intensity at longer wavelength. These results indicate that during the spectra determination in alcoholic solution the pyrazolones existed in at least two tautomeric forms; one with a carbon nitrogen double bond, formula I or II, and the other with the carbon carbon double bond as shown in formulas II and III.

Infrared Absorption Spectra

The infrared absorption spectra of the pyrazolones were determined with a Perkin-Elmer spectrometer. The results obtained are given in Tables IV, V, and VI and some data are plotted in Figs. 4, 5, and 6.

Because of the three tautomeric structures theoretically possible for the 1,3,4-trisubstituted pyrazolones, they may be expected to give rise to very complicated infrared spectra. The problem of interpretation of those spectra

TABLE IV

INFRARED ABSORPTION MAXIMA OF 1-*o*-CHLOROPHENYL-3-PHENYL-4-MONOALKYL-5-PYRAZOLONES

R	Absorption bands, cm.$^{-1}$					
H	1640	1565	1515	1345	1260	1165
CH_3	1645	1575	1535	1315	1265	1120
C_2H_5	1630	1580		1310	1255	1120
C_3H_7	1635	1590	1570	1315	1230	1115
C_4H_9	1630	1575		1325	1250	1125
C_5H_{11}	1640	1610	1580	1310	1250	1120
C_6H_{13}	1630	1575		1315	1240	1170
C_7H_{15}	1640	1610	1575	1325	1255	1125
C_8H_{17}	1640	1615	1580	1315	1250	1125
C_9H_{19}	1640	1610	1585	1320	1250	1120
$C_6H_5CH_2$	1630	1575	1310	1255	1215	1110

TABLE V

INFRARED ABSORPTION MAXIMA OF 1-*m*-CHLOROPHENYL-3-PHENYL-4-MONOALKYL-5-PYRAZOLONES

R	Absorption bands, cm.$^{-1}$					
H	1710	1590	1490	1320	1290	1120
CH_3	1650	1590	1500	1325	1245	
C_2H_5	1650	1590	1480	1330	1230	1120
C_3H_7	1640	1585	1480	1315	1230	1125
C_4H_9	1660	1590	1480	1310	1210	1120
C_5H_{11}	1700	1590	1500	1300		1130
C_6H_{13}	1675	1575	1420	1375	1230	1120
C_7H_{15}	1680	1580	1420	1320	1250	1125
C_8H_{17}	1640	1580	1415	1325	1250	1120
C_9H_{19}	1640	1580	1420	1320	1250	1120
$C_6H_5CH_2$	1640	1575	1490	1310	1230	1110

TABLE VI

INFRARED ABSORPTION MAXIMA OF
1-*p*-CHLOROPHENYL-3-PHENYL-4-MONOALKYL-5-PYRAZOLONES

R	Absorption bands, cm.$^{-1}$					
H	1700	1590	1432	1332	1177	1120
CH_3	1660	1590	1485	1330	1183	
C_2H_5	1700	1605	1562	1490	1162	1090
C_3H_7	1695	1580	1484	1320	1160	1130
C_4H_9	1630	1575	1410	1310	1212	1115
C_5H_{11}	1710	1590	1492	1320	1185	1100
C_6H_{13}	1640	1570		1310	1175	1120
C_7H_{15}	1650	1575		1315	1180	1115
C_8H_{17}	1660	1580	1410	1310	1250	1120
C_9H_{19}	1635	1580	1405	1318	1250	1120
$C_6H_5CH_2$	1640	1575		1310	1250	1105

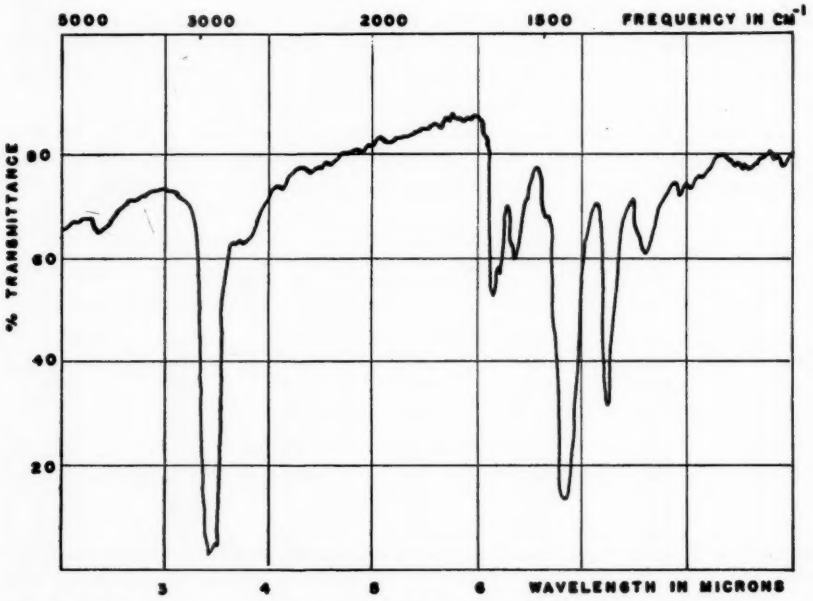


FIG. 4. Infrared absorption spectrum of 1-*o*-chlorophenyl-3-phenyl-4-methyl-5-pyrazolone.

is difficult since few reference spectra for pyrazolones are available in the literature. The absorption spectrum of the 3-methyl-5-pyrazolone has been reported by Randall and his co-workers (10) and he only gave an assignment for a cyclic C=N band. The infrared spectra of several 4-monosubstituted-1,3-diphenyl-5-pyrazolones were determined by Gagnon, Boivin, and Paquin (6). Bond assignments were made tentatively for the C=O group at 1700 cm.$^{-1}$ and the C=N group at 1600 cm.$^{-1}$. Gagnon, Boivin, MacDonald, and Yaffe (5) have studied the absorption spectra of 2-substituted-3-hydroxy-5-pyrazolones and assigned a band in the region of 3300 cm.$^{-1}$ to an OH group. The presence of

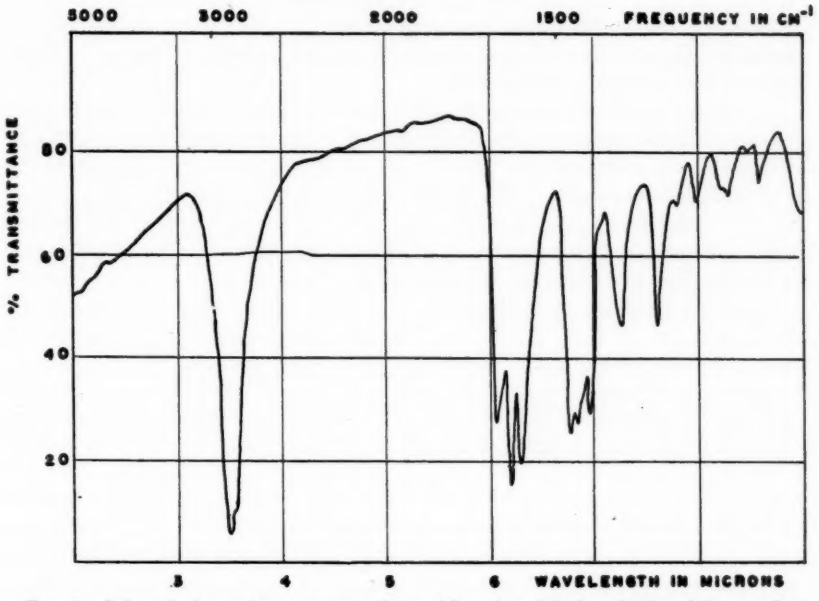


FIG. 5. Infrared absorption spectrum of 1-*m*-chlorophenyl-3-phenyl-4-butyl-5-pyrazolone.

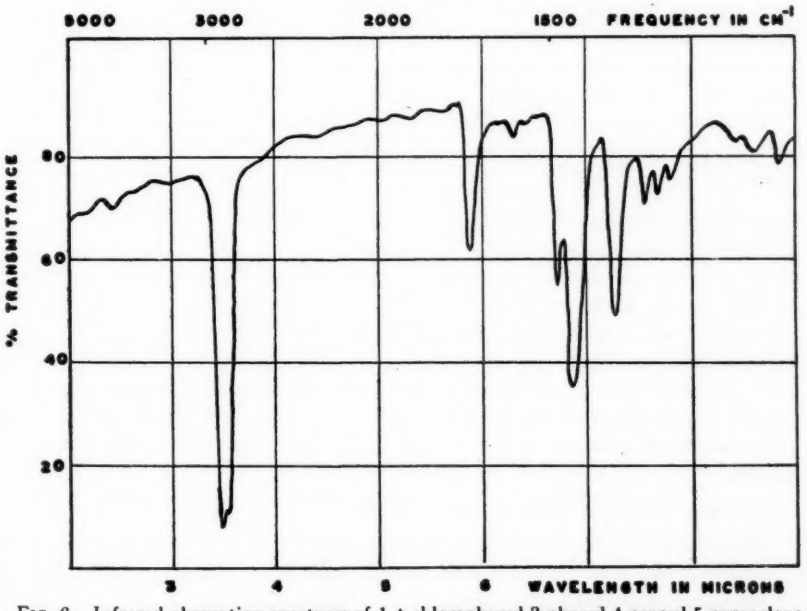


FIG. 6. Infrared absorption spectrum of 1-*p*-chlorophenyl-3-phenyl-4-propyl-5-pyrazolone.

that group was also proved by chemical methods. Another band occurring in the region of 1670–1700 cm.$^{-1}$ was attributed to a C=O group. A third band in the region of 1590–1680 was attributed to a C=N band as suggested by Randall (10) after studying many cyclic nitrogen compounds such as thiazolidines, imidazolines, and pyridines.

In the present investigation band assignments can be made for two functional groups. An absorption band of high intensity occurring in the region of 1630–1710 cm.$^{-1}$ is attributed to a C=O group. Randall and his co-workers have assigned an absorption band in this region to the carbonyl group for the lactams; therefore it is reasonable to do the same for pyrazolones. Furthermore the absence of a band in the region of 3300 cm.$^{-1}$ characteristic of an OH group is another proof for the presence of a carbonyl group.

A second band occurs in the double band region and absorbs in the range of 1565–1610 cm.$^{-1}$. This band is attributed to a C=N group as suggested by Randall after having studied many cyclic nitrogen compounds.

From those results, one might conclude that in the solid state the pyrazolones prepared have the form CH—C=N while in alcoholic solution this form is in equilibrium with its tautomer C=C—NH.

EXPERIMENTAL PART*

1-o-Chlorophenyl-3-phenyl-4-alkyl-5-pyrazolones

A mixture of ethyl-α-monosubstituted-α-benzoylacetate (0.02 mole) and *o*-chlorophenylhydrazine hydrochloride (0.02 mole) was heated under reduced pressure for three hours at 110–120° C. The red viscous liquid obtained was cooled and dissolved in sodium hydroxide (5%, 200 ml.). The solution was filtered and washed with ether until the extracts were no longer colored. The alkaline solution was cooled and acidified with acetic acid (50%). The precipitate obtained was recrystallized from alcohol or petroleum ether. The pyrazolones are listed together with their melting points, analyses, and ultraviolet absorption spectra in Table I and the infrared data are given in Table IV. Typical ultraviolet absorption curves are plotted in Fig. 1 while some infrared data are plotted in Fig. 4.

1-m-Chlorophenyl- and 1-p-Chlorophenyl-3-phenyl-4-monosubstituted-5-pyrazolones

A mixture of ethyl-α-monosubstituted-α-benzoylacetate (0.02 mole) and *m*-chlorophenyl- or *p*-chlorophenyl-hydrazine was heated under reduced pressure for three hours at 110–120° C. and then for one hour at 170–180° C. The red viscous liquid was treated in a manner similar to that used to obtain the 1-*o*-chlorophenylpyrazolones. The melting points, analyses, and ultraviolet absorption maxima are given in Tables II and III. The infrared absorption bands are given in Tables V and VI and some data are plotted in Figs. 5 and 6.

Preparation of Ethyl Benzoylacetate-α-C^{14}

Magnesium (5.6 gm., 0.23 mole) was placed in a flask equipped with a condenser and a mechanical stirrer. Ethyl malonate (32 gm., 0.20 mole) and

*All melting points are uncorrected.

ethyl malonate-2-C^{14} (10 mgm. containing 0.1 mc. of activity) were added. The reaction was initiated with a few drops of carbon tetrachloride and when it subsided, dried ether (75 ml.) was introduced. After all the magnesium had reacted, benzoyl chloride (31 gm., 0.22 mole) was added dropwise while the mixture was refluxed and stirred. When the reaction was completed the solution was cooled and diluted with water (100 ml.). The compound $C_6H_5COCH(CO_2C_2H_5)_2.3H_2O$ crystallized from ether. That substance was decomposed with dilute sulphuric acid. The product was extracted with ether, dried, and distilled. B.p.: 185–190° C. at 12 mm. The benzoyl malonate-2-C^{14} was decarboxylated by steam distillation in dilute sulphuric acid solution. The distillate was neutralized with sodium bicarbonate, extracted with ether, dried, and distilled. B.p.: 113–116° C. at 2 mm.

1-o-Chlorophenyl-3-phenyl-5-pyrazolone-4-C^{14}

Ethyl benzoylacetate-α-C^{14} (3.84 gm., 0.02 mole) was heated with *o*-chlorophenylhydrazine hydrochloride (3.58 gm., 0.02 mole) during three hours at 110–120° C. The product of the reaction was dissolved in sodium hydroxide solution and purified by several extractions with ethyl ether. Acidification with acetic acid (50%) gave the labelled pyrazolone, which was crystallized from ethyl alcohol. Yield, 3.3 gm., 61%.

1-m-Chlorophenyl- and 1-p-Chlorophenyl-3-phenyl-5-pyrazolones-4-C^{14}

A mixture of ethyl benzoylacetate-α-C^{14} (1.92 gm., 0.01 mole) and *m*-chlorophenyl- or *p*-chlorophenyl-hydrazine (1.43 gm., 0.01 mole) was heated under reduced pressure for three hours at 110–120° C. and then for one hour at 170–180° C. The product obtained was treated in a manner similar to that used to obtain the 1-*o*-chlorophenylpyrazolone. The chemical yields varied from 60 to 70%, after purification from ethyl alcohol.

Combustion of C^{14} Products, Plating, and Counting

Samples weighing about fifteen milligrams were completely transformed into carbon dioxide by burning them in a closed system in the presence of oxygen. The carbon dioxide evolved was absorbed in two traps containing carbonate-free sodium hydroxide. After some ammonium chloride was added, the carbonate was precipitated with barium chloride. The barium carbonate was transferred to an aluminum counting plate by centrifuging. Sufficient barium carbonate was added to each plate to ensure "infinite thickness". When dry, the samples were counted using an end window Geiger-Müller counter. The results obtained are given in Table VII.

TABLE VII

COUNTING DATA OF 1-CHLOROPHENYL-3-PHENYL-5-PYRAZOLONES-4-C^{14}

1-Chloro	Weight, mgm.	Total count	Time, min.	Thickness, mgm./cm.^{2}	Net activity, c./min.
Ortho	13.1	20758	25	30.4	801
Meta	13.6	23082	25	30.8	894
Para	13.9	21896	25	31.0	847

Ultraviolet Absorption Spectra

The ultraviolet absorption spectra of the pyrazolones were taken on a Beckman Spectrophotometer Model DU. The method has been previously described (4). The solvent used was ethanol (95%). The results are listed in Tables I, II, and III and some of the data are plotted in Figs. 1, 2, and 3.

Infrared Absorption Spectra

All the infrared absorption spectra of the pyrazolones were obtained with a Perkin-Elmer Model 21 double-beam null principle recording spectrophotometer. The method has been described in the literature (6). The spectra covering the range 5000–1100 cm.$^{-1}$ only are given in Figs. 4, 5, and 6. The curves all show the four specific bands of nujol. The other absorption bands are listed in Tables IV, V, and VI, and have been discussed in the theoretical part.

ACKNOWLEDGMENTS

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REACTION OF ALDOSES AND KETOSES WITH LEAD TETRAACETATE[1]

By A. S. Perlin and Carol Brice

ABSTRACT

Lead tetraacetate is highly selective for oxidation of α-hydroxy-hemiacetal groups and hence most readily attacks cyclic forms of the sugars. The reaction proceeds stepwise; the hemiacetal α-glycol being cleaved and the monoester of a correspondingly shorter-chained sugar formed. After cyclization the new sugar in turn is oxidized at the hemiacetal α-glycol group to yield a diester of a still-lower-order member of the series. In this manner D-glucose first yields mono-*O*-formyl-D-arabinose and then di-*O*-formyl-D-erythrose. Similarly, D-fructose is degraded to a glycolate ester of D-erythrose and finally to a formate–glycolate diester of D-glyceraldehyde. Some relatively rare sugars thus may conveniently be prepared directly from abundant higher-order members of the series. The reactions appear to involve preferential attack of the furanose form of a sugar rather than of the normally-predominant pyranose form, or possibly migration of ester groups towards the reducing end of the sugars.

Criegee noted that D-glucose rapidly consumes about three moles of lead tetraacetate in warm acetic acid without concurrent production of formaldehyde (5). He suggested that the apparent failure of the oxidant to attack the 5,6-glycol group was due to the presence of the 1,5-hemiacetal oxygen bridge, and that the sugar must therefore be oxidized as a ring, rather than in the open-chain form. According to Hockett and Zief (14) D-glucose at 20°C. quickly consumes only two moles of lead tetraacetate and further oxidation is very slow. If the sugar exists in solution predominantly as D-glucopyranose (I) it would be expected to consume at least three moles of oxidant, as had been found by Criegee, and possibly yield 2-*O*-formyl-D-glyceraldehyde (II). For the related oxidant, periodic acid, this possibility has been verified recently by Schöpf and Wild (28) who isolated D-glyceraldehyde monoformate by treating D-glucose with a limited quantity of periodate. In contrast to these results, Abraham (1) reported that D-glucose in warm aqueous acetic acid is completely but slowly degraded by lead tetraacetate to five moles of formic acid and one mole of formaldehyde, the reaction being recommended for radioactive-carbon assay. Hence the literature records at least three different sets of data for the reaction of D-glucose with lead tetraacetate. These variations are attributable most probably to the differences in the oxidation conditions employed but they invited further examination of the reaction.

The results of Hockett and Zief were of particular interest since the consumption of only two moles of oxidant would be expected to afford a derivative of a tetrose, rather than of a triose. Accordingly, D-glucose, in glacial acetic acid or in acetic acid containing 1–2% of water (2), was treated at room temperature with excess lead tetraacetate. Two moles of oxidant were consumed within three minutes and oxidation proceeded subsequently at a

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markedly slower rate reaching a value of 2.1 moles in one hour; the rapidity of the reaction may be contrasted with the relatively slow oxidation rates of polyols (12) and glycosides (13). The product of the initial rapid oxidation, isolated as a sirup in 95% yield, was strongly reducing, very soluble in ethyl acetate, and it absorbed strongly in the infrared region at about 1170 cm.$^{-1}$, characteristic of formate esters (31). In agreement with the latter result two moles of formic acid were liberated when the compound was hydrolyzed with acid or alkali. Acid hydrolysis afforded D-erythrose as the sole sugar (23), characterized by hydrogenation to give erythritol, by oxidation to give D-erythrono-γ-lactone, by formation of a 2,5-dichlorophenylhydrazone, and by comparison of its specific rotation, infrared absorption spectrum, and paper chromatographic behavior with those of authentic D-erythrose. The product of the oxidation of D-glucose with lead tetraacetate is therefore a diformate ester of D-erythrose.

The position of the formate substituents has not been definitely established, in part because of the groups' high instability. For example, formic acid is released spontaneously when the compound is dissolved in water, and the ester groups are removed when the compound is hydrogenated in alcohol at room temperature with platinum oxide (23). This instability thus adds to the difficulty so often encountered in attempting to determine the position of acyl substituents. Because the compound is so very slowly oxidized by lead tetra-

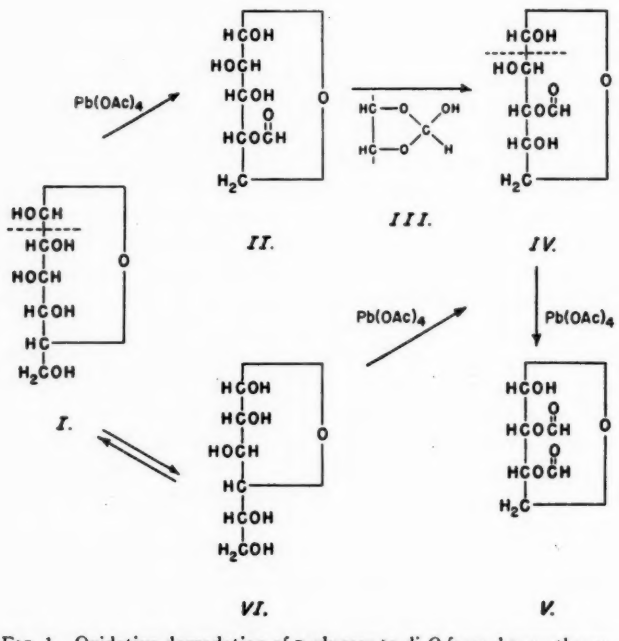

FIG. 1. Oxidative degradation of D-glucose to di-*O*-formyl-D-erythrose.

acetate one formate group is most likely at the 2-position; the second ester group, however, could be on either carbon 3 or 4.

Degradation of D-glucose to a 2,3- or 2,4-diformate ester of D-erythrose may occur in one or both of at least two different ways. According to one of these possibilities (Fig. 1) D-glucopyranose (I) is oxidatively cleaved at the hemiacetal α-glycol group to yield 4-*O*-formyl-D-arabinose (II). Acyl-migration of the formate group to the 3-position (IV) then takes place, perhaps via the orthoacid (III). The newly-formed hemiacetal α-glycol of the pentose consumes a second mole of oxidant to yield 2,4-di-*O*-formyl-D-erythrose, or, if a second acyl-migration follows to permit ring closure, 2,3-di-*O*-formyl-D-erythrose (V). Several known instances of acyl-migration (see 30) provide precedence for this suggested route. However, little is known of the behavior of formate groups because very few sugar formates have been prepared and characterized. The great rapidity of the oxidation as compared with the rates of reaction of, for example, polyols or glycosides may be accommodated by this route if the oxidation proceeds by an ionic mechanism (4, 18) since the hemiacetal α-glycol would be the prime site of carbonium-ion formation.

An alternative route could be depicted by assuming that α-D-glucofuranose (VI), although present perhaps in small proportion, is oxidized preferentially. Thus oxidation of the α-hydroxy-hemiacetal group, accompanied by displacement of the pyranose–furanose equilibrium, would yield 3-*O*-formyl-D-arabinose (IV) directly, which as a furanose sugar in turn would be degraded to (V). The possibility that the furanoid form of the sugar is oxidized preferentially finds support in the fact that the five-membered furan ring is nearly planar and the hemiacetal α-glycol group of α-D-glucofuranose is therefore more nearly able to assume a true *cis* (0°) position. Reeves (26) has shown that compounds having true *cis*-α-glycols, such as 2,3-dihydroxy tetrahydrofuran, react extremely rapidly with lead tetraacetate and may, in fact, be titrated with the reagent in the presence of ethylene glycol or methyl glycopyranosides containing a *cis*-α-glycol group. He noted, further, that reducing sugars can interfere with the titration since they presumably may form furanose rings containing true *cis*-α-glycol groups. The lead tetraacetate – glycol reaction possesses second order kinetics (6, 25) and therefore should require a relatively high concentration of the furanose modification to account for the observed rate of oxidation of D-glucose. However, the position of the pyranose–furanose equilibrium in acetic acid solution is not known, but the great rapidity of the interconversion between the two forms (17) might be sufficient to accommodate displacement of the equilibrium in favor of the latter as oxidation proceeds. This may be true particularly for acetic acid, in which mutarotation is extremely rapid. Thus, it was not possible to observe the changes in rotation when α- or β-D-glucose, dissolved quickly in a minimum quantity of water, was taken up in acetic acid; the specific rotation in each solution had reached an equilibrium value of $[\alpha]_D$ +70° before the polarimeter reading could be made.

From Reeves' data (26) it may be inferred that reducing sugars are titratable in the presence of substances such as ethylene glycol. If, then, α-D-gluco-

furanose is the species oxidized, the hemiacetal α-glycol should be preferred as the point of attack rather than the 5,6-glycol group. Once D-arabinose formate is produced the new hemiacetal ring, either pyranose or furanose, could in turn prevent oxidation of the terminal glycol group. The failure of methyl α-D-mannofuranoside to yield formaldehyde when oxidized with lead tetraacetate was explained in a similar manner (5). On the other hand, absence of oxidation at the 5,6-glycol group of D-glucose could be regarded as evidence favoring the interpretation that D-glucopyranose is the form involved in the reaction, with the hemiacetal ring at carbon-5 preventing attack.

Considering these possibilities further the oxidation behavior of D-*glycero*-D-*gulo*heptose and D-*erythro*-D-*gala*octose was examined. Even in the pyranose form the heptose contains a free terminal 1,2-glycol group and the octose, a 1,2,3-triol group. Thus if a pyranose ring in glucose is required to explain the results just discussed, cleavage of the terminal groups of the higher sugars would be expected. Like D-glucose, the heptose and octose also rapidly consumed two moles of lead tetraacetate and a relatively slow reaction then followed (Fig. 2). When two moles of oxidant had been consumed by the heptose, arabinose was the only product detected chromatographically and the amount (colorimetrically determined) was one mole per mole of heptose. These results corresponded to quantitative removal of carbon atoms 1 and 2 without simultaneous attack at carbons 6 and 7. In 24 hr. reaction time the consumption of oxidant had increased to 3.4 moles per mole but arabinose was still the only product detectable on the chromatogram and the quantity present was now 0.65 moles per mole. The 0.35 moles of pentose formate which appeared to have been completely oxidized in the interim accounted for the extra 1.4 moles of oxidant consumed (i.e., 4 moles per mole of pentose $\times 0.35$). Similarly, the octose yielded the expected glucose on oxidation with two moles of lead tetraacetate and glucose was still present when consumption of oxidant reached four moles per mole. Therefore, it was evident that the terminal glycol groups did not require protection from the original pyranose ring in order to escape oxidation, and the reactions could be depicted in the same general manner as already indicated for D-glucose (Fig. 1). Accordingly the product from the heptose would be 2,3- or 2,4-di-*O*-formyl-D-arabinose and from the octose, 2,3- or 2,4-di-*O*-formyl-D-glucose and further oxidation could result from ring opening and slow hydrolysis of ester groups followed by complete oxidation of the compounds.

In addition to D-glucose other aldoses also rapidly consume two moles of lead tetraacetate (Fig. 3), although a further relatively fast oxidation occurs with some of the compounds. The reaction rates illustrated in Fig. 3 have been greatly reduced relative to those in Fig. 2 by employing a reaction temperature of 0°C. in a mixture of acetic and propionic acids, so that the early portion of the reaction could be examined; the consumption of oxidant by each sugar at room temperature in acetic acid reaches approximately the same level as at the lower temperature. In no instance is a consumption of three moles, expected of a pyranose sugar, attained. Moreover, there is a wide diversity in

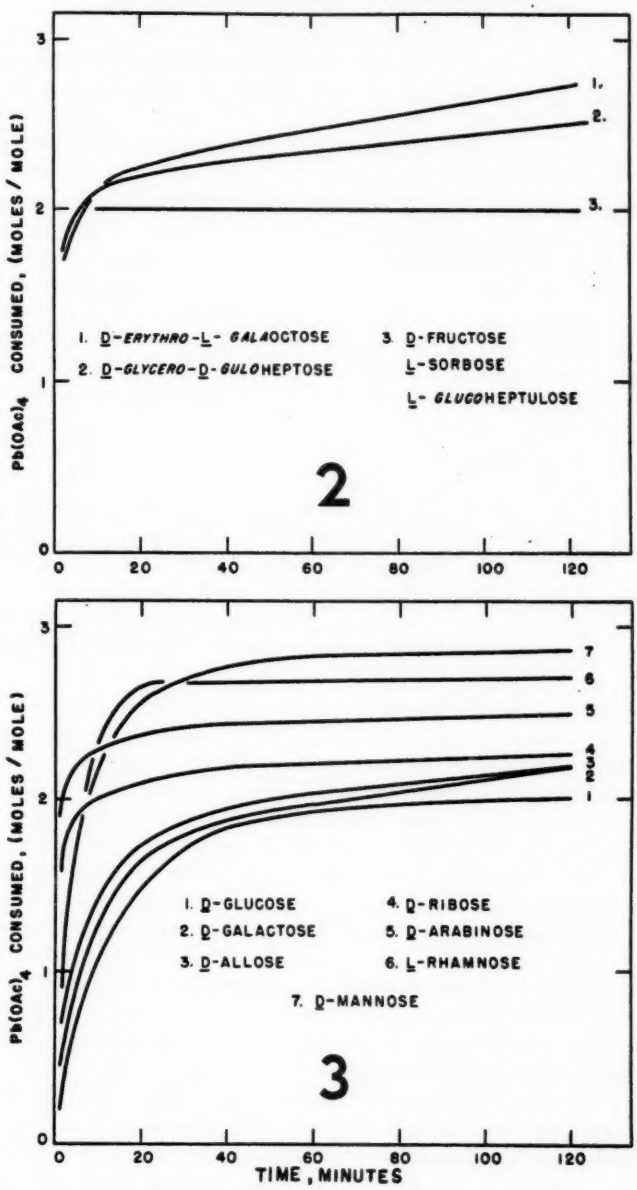

FIG. 2. Rates of lead tetraacetate consumption at room temperature (*ca.* 25°C.).

FIG. 3. Rates of lead tetraacetate consumption for aldoses at 0°C.

the rates of oxidation. These differences have shown no obvious correlation with structural differences between the compounds nor with the predicted stabilities from Reeves' measurements of fixed pyranose rings of the sugar glycosides (27).

Despite these variations the oxidations in general appear to follow a pattern similar to that already described for D-glucose with departures occurring in differing degree. For example, the oxidation of L-arabinose with two moles of lead tetraacetate affords a 90% yield of a sirupy product which from analysis appears to be a triose containing approximately 1.5 formate ester groups, and which on hydrolysis gives L-glyceraldehyde (23), characterized as the dimedon derivative. This triose formate again indicates a cleavage of carbon atoms 1 and 2 from the reducing end of the parent aldose and its production, in part, may be described by the same type of reaction sequences as presented above. The oxidation of D-galactose even more closely resembles the reaction with D-glucose. Treatment with two moles of lead tetraacetate gives a product which contains approximately 1.8 ester groups and which on hydrolysis yields D-threose, characterized as tri-*O*-acetyl-α-D-threose, by hydrogenation to D-threitol, and as a 2,5-dichlorophenylhydrazone. The specific rotation of the D-threose is about $[\alpha]$ $-12°$, which is also the value assigned by Hockett and co-workers (10, 11), who prepared the compound by other methods, but different from Freudenberg's value of $+19°$ (7). By consuming 2.7 moles of lead tetraacetate, D-mannose appears to differ most markedly from D-glucose. It will be seen, however, that in some respects the oxidations of these two compounds are closely similar.

To account for the conversion of a hexose to a tetrose containing two formate ester groups it has been necessary to assume that the oxidation proceeds *via* a pentose monoformate. It was expected therefore that on treatment with somewhat less than two moles of lead tetraacetate a hexose should yield the related pentose, which would constitute a new procedure for the stepwise shortening of the carbon chain of sugars. Accordingly, when D-glucose-1-C^{14} was oxidized with one mole of lead tetraacetate (21) the products found on paper chromatograms after precipitation of lead as the oxalate were unoxidized D-glucose (radioactive by radioautography), a compound which gave color tests for pentoses but which travelled more rapidly than the pentoses (radioactive), and a third compound which travelled at a rate similar to that of D-erythrose diformate (radioactive). After acid hydrolysis to remove ester groups the latter two compounds were no longer radioactive and travelled on the paper chromatogram at the same rate as arabinose and erythrose, respectively. The pentose, subsequently isolated and characterized as D-arabinose, was undoubtedly therefore the expected monoformate ester but the yield was only about 15%. Possibly this low yield is obtained because D-arabinose is much more rapidly oxidized than is D-glucose (Fig. 3) and hence is converted to D-erythrose instead of being accumulated. Application of this reaction to studies employing C^{14}-labelled sugars has been suggested (21).

D-Mannose is oxidized much more rapidly than glucose and since, like D-glucose, it also is degraded to D-arabinose the amount of the pentose oxidized

further to D-erythrose is proportionately less (Fig. 2). When treated with 0.8 moles of lead tetraacetate, for example, D-mannose gave D-arabinose in about 35% yield; since 35% of the D-mannose remained unoxidized the actual conversion of hexose to pentose was greater than 50%. A 35% yield of pentose was also obtained in the degradation of D-galactose to D-lyxose. On oxidation with one mole of lead tetraacetate D-altrose and D-allose are degraded to D-ribose, the yields being approximately 70% and 20%, respectively. The difference between these two hexose epimers appears again to be related to differences in oxidation rates; D-altrose consumes lead tetraacetate at a rate close to that of D-mannose, attaining an oxidation level of 2.6 moles per mole, but D-allose is oxidized much more slowly (Fig. 3). These results support the suggestion that the lead tetraacetate reaction involves an initial attack at the α-hydroxy-hemiacetal group, but also indicate that the conformation of the rest of the molecule markedly affects the rate of oxidation.

According to Hockett and co-workers (14, 13) D-fructose also consumes only two moles of lead tetraacetate and at a rate close to that for D-glucose. In the present investigation this result was confirmed and similar results were also obtained with L-sorbose and L-*gluco*heptulose (Fig. 3). The product from the oxidation of D-fructose with two moles of lead tetraacetate, isolated in 95% yield, gave on hydrolysis approximately one mole each of formic acid, glycolic acid, and D-glyceraldehyde (24). Similarly, the oxidation of L-sorbose produced a formic acid – glycolic acid diester of L-glyceraldehyde (24). The formation of these diesters from ketohexoses may be described by reaction sequences (Fig. 4) similar to those considered earlier for aldose oxidations. Assuming β-D-fructofuranose (VII) to be the species chiefly concerned in the

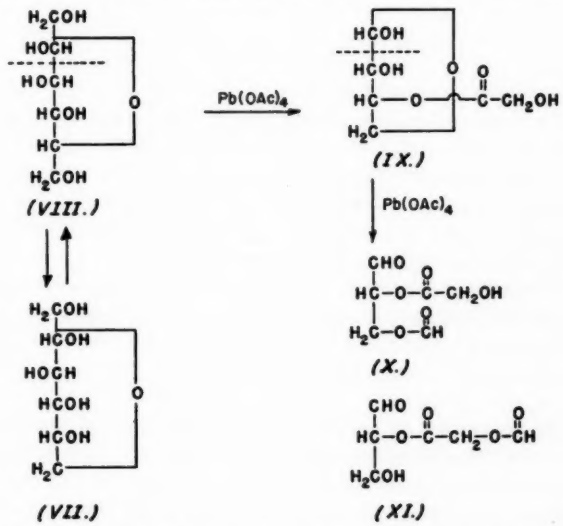

FIG. 4. Oxidative degradation of D-fructose to a formate–glycolate diester of D-glyceraldehyde.

reaction, the initial attack cleaves the hemiacetal α-glycol group to yield 3-*O*-glycolyl-D-erythrose (VIII), which in turn is degraded to 3-*O*-formyl-2-*O*-glycolyl-D-glyceraldehyde (IX). Alternatively compounds VIII and IX may be produced from D-fructopyranose by an initial acyl-migration of the glycolic acid ester group from carbon atom 4 of D-erythrose to carbon atom 3. Since the glycolate ester group itself contains a primary hydroxyl the latter conceivably could also accommodate the formate ester group, as in X.

A mechanism for the reaction of aldoses and ketoses with lead tetraacetate cannot be described at present with certainty. It is seen, however, that the oxidation is highly selective for α-hydroxy-hemiacetal groups, and hence involves cyclic forms of the sugars almost exclusively as first proposed by Criegee (5). The reaction proceeds stepwise with cleavage of the hemiacetal α-glycol group and formation of a monoester of a new sugar having a shorter carbon-chain. After cyclization the new sugar in turn is attacked oxidatively at the hemiacetal α-glycol group to yield a diester of a still-lower-order member. No racemization appears to be involved in these reactions. With aldoses the oxidation tends to stop after the consecutive scission of carbons 1 and 2, and to yield a diformate ester of the corresponding sugar with two carbon atoms less. The initial oxidation of ketoses involves cleavage of carbons 1 and 2 together as glycolic acid and the reaction then stops after carbon 3 is converted to formic acid, the final product being a formate–glycolate diester of the corresponding sugar having three carbon atoms less. Because of these characteristics the reaction constitutes a means for the stepwise degradation of sugars and thus provides a simple method for preparing some relatively rare carbohydrates directly from higher-order members of the series.

EXPERIMENTAL

Lead tetraacetate was prepared according to the procedure recommended by Vogel (32), or was obtained commercially (Matheson Co., Inc.), and was kept moist with acetic acid during storage. The weight taken for a reaction was based on the predetermined oxidant content of the wet material. If the lead tetraacetate was dark it was recrystallized from acetic acid before use, or it was dissolved in acetic acid and the solution was filtered and added directly to the aqueous acetic acid solution of the sugar.

Cellulose chromatography was carried out according to Hough *et al.* (16) using butanol, half-saturated with water, as the developing solvent. The eluate was first passed through a Seitz bacterial filter and after distillation of the solvent the sugar sirup was extracted with water and clarified with charcoal.

Pentose was estimated colorimetrically by the orcinol method (20). The lead was precipitated with excess oxalic acid, the acetic acid was distilled, and the residue was taken up in water and its pentose content estimated. The values obtained were not corrected for possible interference by other sugars present.

Solutions were concentrated *in vacuo* at 35–40°C.

Measurement of Oxidation Rates

Measurements were made both at room temperature (about 25°C.) and at 0°C. In experiments carried out at room temperature the sugar (25–30 mgm.), dissolved in 0.4 ml. of water (2), was taken up in 20 ml. of acetic acid. At zero reaction time 400 mgm. of lead tetraacetate in 20 ml. of acetic acid was added to the sugar solution, a reagent blank being run simultaneously. Five-milliliter aliquots of the reaction mixture were withdrawn at desired intervals and treated with 10 ml. of stopping solution (10 gm. of potassium iodide and 50 gm. of sodium acetate in 100 ml. of water (4)), and the iodine released was titrated with sodium thiosulphate. Some reactions were carried out in glacial acetic acid, the sugar being first finely ground and dissolved directly.

*Di-O-formyl-*D*-erythrose*

D-Glucose (1.5 gm., 8.3 mM.) dissolved in 3 ml. of water was taken up in 150 ml. of acetic acid. Lead tetraacetate (7.7 gm., 17.4 mM.) was added to the rapidly stirred sugar solution over a period of three to four minutes. When the oxidant had dissolved, oxalic acid dihydrate (1.9 gm.) dissolved in acetic acid was added, and the suspension was stirred for an additional 30 min. The precipitate was filtered and washed with acetic acid and the filtrate was concentrated to a volume of a few milliliters. Ethyl acetate was added and the precipitate which formed was triturated with several portions of ethyl acetate. The extracts were combined, filtered, and concentrated to a sirup which was further purified twice by extraction into ethyl acetate. The product (1.3 gm.) was a clear, pale yellow oil, $[\alpha]_D^{25}$ +20° (*c*, 3, water) dropping slowly owing to hydrolysis of the ester groups. The infrared absorption spectrum showed very strong absorption at 1725 cm.$^{-1}$ and at 1170 cm.$^{-1}$, characteristic of formate esters (31). A sample of the oil (7.4 mgm.) required 8.35 ml. of 0.010 *N* sodium hydroxide in 10 min. reaction time at room temperature, equivalent to 2.0 formate ester groups per mole. Another sample (21.5 mgm.) was slowly titrated with 0.05 *N* sodium hydroxide to a phenolphthalein end point. The solution was then acidified with sulphuric acid and extracted continuously with ether for four hours; the ether extract required 5.20 ml. of 0.048 *N* sodium hydroxide, equivalent to 2.0 formate ester groups per mole. The formic acid content of the ether extract was determined by lead tetraacetate – potassium acetate titration (22) to be equivalent to 2.0 moles per mole. On hydrolysis with dilute acid the compound gave D-erythrose in 90% yield (23).

One sample of the ester which had been kept at 3°C. for several months partially crystallized but the crystalline material could not adequately be separated from the sirup. The infrared absorption spectrum of a specimen from which the majority of sirup had been separated by tiling was similar to that of the sirup but the absorption bands were sharper and now appeared as doublets. The crystalline material failed to induce crystallization of other preparations of the diester and may possibly have been a monoester formed by slow hydrolysis during storage.

D-*Threose Formate Ester*

D-Galactose was treated with 2.05 molar equivalents of lead tetraacetate and the reaction mixture was worked up as described above for the preparation of di-*O*-formyl-D-erythrose; 1.5 gm. of D-galactose yielded 1.2 gm. of sirup, $[\alpha]_D^{27}$ +15° (*c*, 3, water) dropping slowly owing to hydrolysis of the ester groups. Alkaline hydrolysis indicated that the compound contained 1.8 formate groups per mole. The infrared absorption spectrum was very similar to that of D-erythrose diformate.

D-*Threose*

Crude D-threose formate, obtained by distillation of the acetic acid but without purification by extraction into ethyl acetate, was taken up in 0.05 *N* sulphuric acid and heated at 45°C. for 18 hr. to hydrolyze the ester groups. The hydrolyzate was neutralized with Dowex-1 resin (bicarbonate form) and was concentrated to a sirup. Weight, 1.68 gm. from 3.0 gm. of D-galactose. Chromatographic examination with the methyl ethyl ketone solvent (3) showed a major component which travelled slightly faster than D-erythrose, and which gave a yellow-brown color and a bright fluorescence under ultraviolet light with aniline oxalate spray (15); a small quantity of a pentose which corresponded on the paper to D-lyxose was also present. A portion of the sirup (1.1 gm.) was chromatographed on a cellulose column and the tetrose fraction was recovered. Weight 0.70 gm., $[\alpha]_D^{25}$ −12.9° (*c*, 2.3, water). Hockett (10) reports $[\alpha]_D$ −12° for D-threose.

D-*Threitol*

D-Threose formate ester (0.79 gm.) was dissolved in 15 ml. of water and was carefully saponified with 1 *N* sodium hydroxide. Sodium borohydride (0.20 gm.) dissolved in 10 ml. of water was added. A negative Fehling's test was obtained in 90 min. reaction time and acetic acid was then added to decompose excess borohydride. Cations were removed with Amberlite IR-120 resin and boric acid by repeated distillation of the sirup with methanol. The product was recrystallized from ethanol. Weight 0.27 gm., m.p. 76–83°C. After two further recrystallizations, m.p. 86–88°C.; $[\alpha]_D^{25}$ −10.6° (*c*, 1, ethanol), $[\alpha]_D^{25}$ +4.2° (*c*, 1, water) in agreement with literature values (8). Calculated for $C_4H_{10}O_4$: C, 39.34%; H, 8.25%; found: C, 39.62%; H, 8.15%.

Dibenzylidene D-threitol, m.p. 225–226°C., was prepared from the compound according to Haskins and co-workers (8).

Tri-O-acetyl-α-D-threose

D-Threose sirup (300 mgm.) was heated at 100°C. for one hour with 15 ml. of acetic anhydride and 0.5 gm. of sodium acetate. The acetic anhydride was distilled *in vacuo*, the residue was extracted with chloroform, and the chloroform extract washed with water. After evaporation of the chloroform the product was recrystallized from alcohol. Weight 115 mgm., m.p. 115–118°C. Two further recrystallizations raised the melting point to 118–120°C., $[\alpha]_D^{25}$ +34.4° (*c*, 2, chloroform). Hockett (10) reports m.p. 117–118°C. and $[\alpha]_D$ +35.5° for D-threose triacetate. Calculated for $C_{10}H_{14}O_7$: C, 48.78%; H, 5.73%; acetyl, 52.4%; found: C, 48.90%; H, 5.69%; acetyl, 52.2%.

D-*Threose 2,5-Dichlorophenylhydrazone*

D-Threose sirup (0.51 gm.), prepared by concentrating an aliquot of the neutral hydrolyzate described above, was dissolved in 20 ml. of methanol in an evaporating dish. 2,5-Dichlorophenylhydrazine (0.75 gm.) was added and the methanol was rapidly evaporated off on the steam bath (procedure of Mandl and Neuberg (19)). The product was taken up in ether, filtered, and the ether was distilled. The residue was dissolved in ethyl acetate, treated with charcoal, and an equal volume of benzene was added. The product (0.60 gm.) had m.p. 103–106°C. Recrystallized twice, m.p. 108–110°C.; $[\alpha]_D^{25}$ +14.4°. A mixed m.p. with D-erythrose 2,5-dichlorophenylhydrazone (m.p. 110–112°C. (23)) was depressed. Calculated for $C_{10}H_{20}O_3N_2Cl_2$: C, 43.03%; H, 4.33%; found: C, 43.08%; H, 4.28%.

Regeneration of D-*Threose from the Hydrazone*

D-Threose 2,5-dichlorophenylhydrazone was treated with benzaldehyde according to the procedure described by Sowden and Fischer (29). The product had $[\alpha]_D$ +21.2° (*c*, 1.5, water) and in addition to the free sugar appeared to contain a glycoside formed presumably during the reflux with alcoholic benzaldehyde. An aqueous solution of the product was acidified with dilute hydrochloric acid and heated on the steam bath for 5–10 min. which effected a sharp decrease in rotation and an increase in reducing power. D-Threose (91 mgm.) was obtained from 280 mgm. of the hydrazone, $[\alpha]_D^{25}$ −11.4° (*c*, 1.8, water). On the paper chromatogram the regenerated D-threose exactly corresponded to the compound isolated by column chromatography.

L-*Glyceraldehyde Formate Ester*

L-Arabinose was treated with 2.1 molar equivalents of lead tetraacetate and the reaction was worked up as described above for the preparation of di-*O*-formyl-D-erythrose. A clear, yellow oil was obtained, 1.2 gm. from 1.5 gm. of L-arabinose. The infrared absorption spectrum of this compound showed strong absorption characteristic of formate ester groups. On saponification the compound consumed alkali equivalent to approximately 1.5 formate ester groups per mole. Hydrolysis of the ester groups in 60% acetic acid afforded L-glyceraldehyde in 83% yield (23).

D-*Arabinose*

D-Mannose (1.0 gm., 5.56 mM.), dissolved in 2 ml. of water, was taken up in 250 ml. of acetic acid. Lead tetraacetate (2.1 gm., 4.73 mM.) was added to the stirred solution; a negative potassium iodide – starch test was obtained within two minutes reaction time. Oxalic acid dihydrate (0.59 gm. in 6 ml. of acetic acid) was added, stirring was continued for an additional 30 min., and the suspension was filtered. The filtrate was evaporated to a sirup which was chromatographed on a cellulose column. Separation of the arabinose from the unoxidized mannose, sometimes difficult by chromatography, was readily achieved in the present instance because the pentose was present in the mixture as the formate ester. Its rate of travel was thus greatly enhanced and it was completely eluted from the column within 24 hr. A fraction containing the D-erythrose formate ester (0.17 gm.) was eluted just ahead of the pentose

and the unoxidized D-mannose (0.34 gm.) was recovered by prolonged elution. In passage through the column the pentose ester was partially hydrolyzed and the product was therefore a mixture of esterified and free sugar. The ester remaining was hydrolyzed by heating in water for three hours on the boiling-water bath, and the final sirup obtained crystallized completely in one to two days. Weight 0.32 gm. Recrystallized from alcohol the product had m.p. 150–152°C., raised by a second recrystallization to 156.5–157.5°C., undepressed by admixture with authentic D-arabinose (m.p. 157°C.). $[\alpha]_D^{27}$ −102.0°. The X-ray diffraction pattern of the compound was identical with that of D-arabinose.

D-Arabinose was prepared also from D-glucose by partial oxidation as described above for D-mannose. The yield of pentose estimated colorimetrically was about 15%, and the product was isolated by chromatography and characterized as the benzoylhydrazone (9), m.p. 185–186°C. The X-ray diffraction pattern was identical with that of D-arabinose benzoylhydrazone.

D-Lyxose

D-Galactose (1.0 gm., 5.56 mM.) was oxidized with 3.4 gm. (7.68 mM.) of lead tetraacetate and the product was worked up as described above under D-arabinose. Prior to chromatography on the cellulose column the formate ester groups were hydrolyzed by heating an aqueous solution of the oxidation products on the boiling-water bath for three hours. D-Threose (0.31 gm.) was eluted first, and 0.19 gm. of unoxidized D-galactose was recovered from the column following the pentose fraction. The middle fraction (0.37 gm.) crystallized completely when seeded with D-lyxose. Recrystallized from alcohol it had m.p. 113–116°C., and mixed m.p. 114–115°C. with authentic D-lyxose (m.p. 116.5°C.). $[\alpha]_D^{25}$ −13.9° (*c*, 2, water).

On acetylation by refluxing with acetic anhydride and sodium acetate the sugar yielded α-D-lyxose tetraacetate, m.p. 94–96°C., undepressed by admixture with an authentic specimen.

3-O-Formyl-2-O-glycolyl-D-glyceraldehyde

D-Fructose was treated with 2.1 molar equivalents of lead tetraacetate as described above for the preparation of di-*O*-formyl-D-erythrose. The ethyl acetate extract afforded a clear, pale yellow sirup, 1.47 gm. from 1.5 gm. of D-fructose. $[\alpha]_D^{25}$ +5.1° (*c*, 1, water) dropping slowly owing to hydrolysis of the ester groups. The infrared absorption spectrum of the compound was similar to that of the formate esters described above, but the carbonyl bands were broader, presumably owing to the contribution of the glycolate ester group. On complete hydrolysis of the ester groups with dilute acid the product gave D-glyceraldehyde in 90% yield (24).

The hydrolyzate obtained from 0.26 gm. of the diester was neutralized by slow addition of Dowex-1 resin (200–400 mesh; bicarbonate form) to the stirred solution and the resin was filtered off and washed. The resin was then shaken with 20 ml. of 1 *N* sulphuric acid for 90 min., filtered and washed well on the filter, and the acid eluate was concentrated to dryness using a dry ice – acetone trap as condenser. Water was added and the distillation resumed,

and the procedure was repeated four times. The formic acid content of the distillate was determined by lead tetraacetate – potassium acetate oxidation (22) to be 1.1 mM.; requires 1.5 mM. After distillation of the formic acid the residue was three times extracted under reflux with ether. Concentration of the ether extract gave glycolic acid, 0.083 gm. (1.09 mM.; requires 1.5 mM.), m.p. 67–72°C. Recrystallized from ether m.p. 72–75°C., undepressed by admixture with authentic glycolic acid.

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EFFECT OF COMPLEXING ON THE HOMOGENEOUS CATALYTIC ACTIVATION OF HYDROGEN BY CUPRIC SALTS[1]

By E. Peters[2] and J. Halpern

ABSTRACT

The effects of various organic and inorganic complex-forming reagents on the homogeneous catalytic activation of H_2 by Cu^{++} in aqueous solution have been determined. The catalytic activities of the cupric complexes decrease in the order: butyrate, propionate > acetate > $SO_4^=$ > Cl^- > H_2O (i.e. the uncomplexed Cu^{++} ion) > glycine, ethylenediamine. The mechanism of the activation process is discussed.

INTRODUCTION

Detailed kinetic studies of the homogeneous catalytic activation of molecular hydrogen in aqueous perchlorate (11) and acetate (4, 2, 10) solutions were described earlier. In view of the fact that the catalytic activity of the undissociated cupric acetate complex was found to be about 120 times greater than that of the uncomplexed cupric ion, it seemed of interest to examine the effect of other complexing agents. In the present paper kinetic studies on the catalytic activation of hydrogen by a number of organic and inorganic cupric complexes are described.

EXPERIMENTAL

Glycine and ethylenediamine were Eastman white label grade products. All other chemicals were of reagent grade. Hydrogen and nitrogen gases were supplied by Canadian Liquid Air Co.

As in previous investigations (10, 11) the rate of hydrogenation of $Cr_2O_7^=$ was used as a measure of the rate of catalytic activation of H_2. The equipment and experimental procedure have been described earlier (10, 11). When the solutions contained chloride, a titanium liner was placed in the autoclave and the stainless steel stirrer, thermocouple well, and sampling tube were replaced by titanium counterparts to avoid corrosion.

To follow the reaction, the solution was sampled periodically and analyzed for $Cr_2O_7^=$, usually by measuring the optical density at 3500 Å with a Beckman DU spectrophotometer. This procedure was not suitable for chloride-containing solutions because of interference from cupric chloride complexes which absorb light of this wave length. $Cr_2O_7^=$ was determined in such solutions by a volumetric method based on adding an excess of $FeSO_4$ and back-titrating with $KMnO_4$. The cupric content of the solutions was determined electrolytically.

In each experiment, prior to introduction of the H_2, the solution was allowed to remain in the autoclave under N_2 for a period of time comparable with the duration of the subsequent reaction. The rate at which $Cr_2O_7^=$ was consumed under these conditions, by side reactions with the complexing reagent or with the metal reaction vessel, was determined and subtracted from the subse-

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quently measured rate of consumption in the presence of H_2 to obtain the rate of reaction with H_2. This correction was usually small, in most cases less than five per cent of the latter rate. Exceptions to this rule were encountered with solutions containing formate and glycine, whose rates of reaction with $Cr_2O_7^=$ under certain conditions were of the same order as that of H_2. The estimation of the latter rate in these systems was therefore subject to appreciable error.

RESULTS AND DISCUSSION

In all the experiments for which results are reported, the rate of disappearance of $Cr_2O_7^=$ was found to be of zero order in the concentration of the latter. Typical rate plots, obtained for various solutions, are shown in Fig. 1 and are

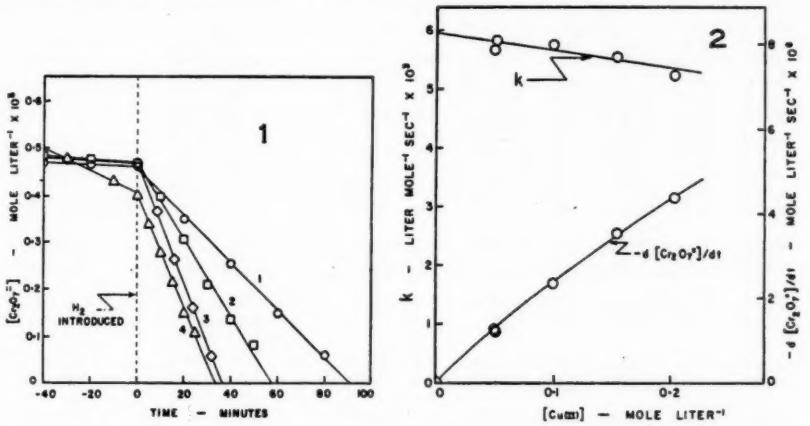


FIG. 1. Typical rate plots for reaction of H_2 with $Cr_2O_7^=$ in presence of various cupric complexes. H_2 partial pressure, 20 atm. 1. Ethylenediamine (0.05 *M* Cu^{++}, 0.20 *M* EDA, pH 4.0, 130° C.). 2. Chloride (0.10 *M* Cu^{++}, 1.0 *M* Cl^-, 110° C.). 3. Sulphate (0.10 *M* Cu^{++}, 0.10 *M* $SO_4^=$, 110° C.). 4. Glycine (0.05 *M* Cu^{++}, 0.05 *M* Gl, pH 3.5, 130° C.).

FIG. 2. Dependence of rate on cupric propionate concentration. 100° C. 20 atm. H_2.

of the same form as those reported earlier for cupric perchlorate (11) and cupric acetate (10).

The reaction between $Cr_2O_7^=$ and H_2 is represented by:

$$Cr_2O_7^= + 3H_2 + 8H^+ \rightarrow 2Cr^{+++} + 7H_2O. \qquad [1]$$

Using this stoichiometric relation, the rate of consumption of H_2 ($-d[H_2]/dt$) was determined from the measured rate of disappearance of $Cr_2O_7^=$ and expressed in terms of an apparent rate constant, k, defined by:

$$-d[H_2]/dt = -3d[Cr_2O_7^=]/dt = k[\text{Cu (II)}]\,[H_2] \qquad [2]$$

where [Cu (II)] is the total cupric ion concentration (including simple and complex forms) in the solution.

A kinetic relation of this form was shown earlier (10, 11) to apply to solutions of cupric perchlorate and cupric acetate. With solutions containing more than one cupric species, k, determined in this manner, obviously represents a weighted average of the rate constants for all the species.

The solution concentration of hydrogen, $[H_2]$, was estimated from the measured partial pressure using available data for the solubility of H_2 in water (12, 14).

Cupric Carboxylate Complexes

Measurements of the rate of reduction of $Cr_2O_7^=$ by H_2 in the presence of various cupric carboxylate salts are reported in Table I.

TABLE I

RATE OF REACTION OF H_2 WITH $Cr_2O_7^=$ IN SOLUTIONS OF VARIOUS CUPRIC CARBOXYLATE SALTS
H_2 partial pressure—20 atm.

Temp., °C.	Solution composition, mole liter^{-1}						Reaction rate	
							$-d[Cr_2O_7^=]/dt$, mole liter^{-1} sec.$^{-1}$	k, liter mole^{-1} sec.$^{-1}$
100.0	0.05	$CuPr_2$;	0.0	NaPr;	0.5	HPr	1.46×10^{-6}	5.75×10^{-3}
100.0	0.05	$CuPr_2$;	0.25	NaPr;	0.5	HPr	1.46×10^{-6}	5.83×10^{-3}
100.0	0.05	$CuPr_2$;	0.5	NaPr;	0.5	HPr	1.61×10^{-6}	6.33×10^{-3}
100.0	0.05	$CuPr_2$;	1.0	NaPr;	0.5	HPr	1.50×10^{-6}	5.94×10^{-3}
100.0	0.05	$CuPr_2$;	0.25	NaPr;	0.2	HPr	1.48×10^{-6}	5.90×10^{-3}
100.0	0.05	$CuPr_2$;	0.25	NaPr;	2.0	HPr	1.83×10^{-6}	7.13×10^{-3}
80.5	0.05	$CuPr_2$;	0.25	NaPr;	0.5	HPr	2.31×10^{-7}	9.16×10^{-4}
90.2	0.05	$CuPr_2$;	0.25	NaPr;	0.5	HPr	5.95×10^{-7}	2.34×10^{-3}
110.0	0.05	$CuPr_2$;	0.25	NaPr;	0.5	HPr	3.50×10^{-6}	1.37×10^{-2}
120.6	0.05	$CuPr_2$;	0.25	NaPr;	0.5	HPr	8.60×10^{-6}	3.32×10^{-2}
100.0	0.026	$CuBu_2$	0.25	NaBu	0.5	HBu	7.70×10^{-7}	5.82×10^{-3}
100.0	0.052	$CuBu_2$	0.25	NaBu	0.5	HBu	1.53×10^{-6}	5.86×10^{-3}
100.0	0.10	$CuFo_2$	0.25	NaFo	0.25	HFo	9.8×10^{-7}	1.8×10^{-3}
100.0	0.10	$CuFo_2$	0.25	NaFo	0.25	HFo	9.6×10^{-7}	1.8×10^{-3}
100.0	0.01	CuSu	0.25	Na_2Su	0.25	H_2Su	1.3×10^{-7}	2.8×10^{-3}
100.0	0.01	CuSu	0.05	Na_2Su	0.60	H_2Su	1.1×10^{-7}	2.2×10^{-3}
100.0	0.01	CuMa	0.125	Na_2Ma	0.015	H_2Ma	5.3×10^{-8}	1.1×10^{-3}
100.0	0.002	CuFu	0.025	Na_2Fu	0.125	H_2Fu	2.7×10^{-8}	2.5×10^{-3}

The results for cupric propionate ($CuPr_2$)* parallel those obtained earlier for $CuAc_2$ suggesting that the mechanism of activation of H_2 in the two systems is essentially the same. Fig. 2 shows that the rate is nearly proportional to the $CuPr_2$ concentration, a slight tendency being apparent for the rate constant, k, to decrease at higher $CuPr_2$ concentrations. A similar effect in the $CuAc_2$ system (10) was attributed to a lowering of the solubility of H_2. The fact that the rate is essentially independent of the concentrations of NaPr and HPr, over a wide range, suggests that the catalytic activity is associated with a fully complexed cupric species. In the case of cupric acetate it has been suggested that the principal active species is the undissociated $CuAc_2$ molecule. By analogy, the active species in the present case is probably $CuPr_2$, although the possibility of participation of other complexes with catalytic activities of the same order as $CuPr_2$ is not excluded.

At 100° C., the catalytic activity of $CuPr_2$ in propionate-buffered solutions ($k = 6.0\times10^{-3}$ liter mole^{-1} sec.$^{-1}$) was found to be about 20% higher than that determined earlier for $CuAc_2$ ($k = 5.0\times10^{-3}$ liter mole^{-1} sec.$^{-1}$) and about

*The following abbreviations are used in this paper: Pr–propionate; Bu–butyrate; Ac–acetate; Fo–formate; Su–succinate; Ma–maleate; Fu–fumarate; Gl–glycinate; EDA–ethylenediamine.

150 times as great as that for the uncomplexed Cu^{++} ion ($k = 4.1\times10^{-5}$ liter mole^{-1} sec.$^{-1}$). Rate measurements for $CuPr_2$ at temperatures between 80° and 120° C. gave a good Arrhenius plot from which an apparent activation energy of 24.8±0.8 kcal./mole and a frequency factor of 2×10^{12} liter mole^{-1} sec.$^{-1}$ ($\Delta S^{\ddagger} = -4.7$ e.u. at 100° C.) were calculated. These values are in good agreement with those reported earlier for $CuAc_2$ (10).

Two experiments with $CuBu_2$ in butyrate-buffered solutions yielded rates identical with those for $CuPr_2$.

Measurements of the rate of activation of H_2 in formate-buffered solutions were subject to large errors because of complications arising from side reactions between formate and $Cr_2O_7^{=}$. The rate at 100° C. ($k = 1.8\times10^{-3}$ liter mole^{-1} sec.$^{-1}$) was about one third that for $CuAc_2$ and the higher carboxylate complexes. This may reflect the fact that complexing between Fo^{-} and Cu^{++} is incomplete, consistent with the higher dissociation constant of formic acid. Similar considerations apply to cupric fumarate and cupric maleate.

The catalytic activity of succinate-buffered cupric solutions ($k = 2.5\times10^{-3}$ liter mole^{-1} sec.$^{-1}$ at 100° C.) was about one half that for $CuAc_2$. It is probable that the CuSu complex has a chelate structure.

Cupric Sulphate

The results for cupric sulphate are summarized in Figs. 3 and 4. It is seen that addition of Na_2SO_4 was found to increase the catalytic activity of Cu^{++}, presumably the result of complex formation.* It has been suggested that only the first stage of association, i.e.

$$Cu^{++} + SO_4^{=} \rightleftharpoons CuSO_4, \qquad [3]$$

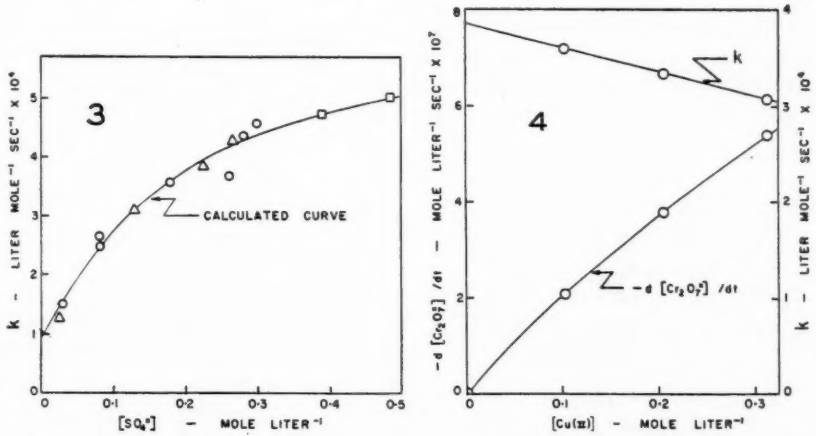


FIG. 3. Dependence of rate on $SO_4^{=}$ concentration. 0.10 *M* Cu^{++}, 20 atm. H_2. 110° C. Experimental points: ○ Total ionic strength held constant at 1.0; $[HSO_4^{-}] = 0$. □ Total ionic strength variable (>1); $[HSO_4^{-}] = 0$. △ $[HSO_4^{-}] + [SO_4^{=}] = 0.32$ *M*.

FIG. 4. Dependence of rate on cupric ion concentration in sulphate solution. 0.25 *M* $SO_4^{=}$, 0.10 *M* HSO_4^{-}, 20 atm.H_2, 110° C.

**Since sodium perchlorate was found to be without effect on the rate of catalytic activation of H_2 by cupric perchlorate, it seems reasonable to interpret the effects obtained on adding other sodium salts in terms of complexing of the anions with Cu^{++}.*

is important (7, 9), and that higher cupric sulphate complexes are not formed to an appreciable extent. The failure of the curve in Fig. 3 to level off suggests that complexing is incomplete at $SO_4^=$ concentration as high as 0.5 M./liter.

Consistent with the above interpretation, it was found that the experimental results could be fitted by a relation of the form:

$$k = (k_1[Cu^{++}] + k_2[CuSO_4])/[Cu\ (II)] \qquad [4]$$

where k_1 and k_2 represent the specific rate constants for the activation of H_2 by Cu^{++} and $CuSO_4$ respectively. k_1 is known from measurements in perchlorate solution (11) and has a value of 1.0×10^{-4} liter mole^{-1} sec.$^{-1}$ at 110° C.

From equation [3], Cu^{++} and $CuSO_4$ are related by

$$[CuSO_4]/[Cu^{++}]\,[SO_4^=] = K \qquad [5]$$

while

$$[Cu^{++}] + [CuSO_4] = [Cu\ (II)]. \qquad [6]$$

The best correspondence between a curve for k, calculated from equations [4], [5], and [6], and the experimental points (shown in Fig. 3) was obtained using the following values: $k_2 = 6.5\times10^{-4}$ liter mole^{-1} sec.$^{-1}$ at 110° C. (i.e. about six times as great as k_1) and $K = 6.7$ liter mole^{-1}. The excellent fit lends support to these values. However, some uncertainty arises through neglect of activity coefficients and of the effect of variable salt concentration on the solubility of H_2. It is of interest that the above value of K, derived from the kinetic results at 110° C., is of the same order as the room temperature value (≈4.5 liter mole^{-1} for ionic strength of unity) estimated by a spectrophotometric method (9).

Cupric Chloride Complexes

The promoting influence of Cl^- on the catalytic activity of Cu^{++} at 110° C. (Fig. 5) is attributed to the formation of cupric chloride complexes. It is probable that in the region above 1 M./liter Cl^-, where the rate levels off, the cupric ion is fully complexed, i.e. exists as $CuCl_4^=$. Hence it seems reasonable

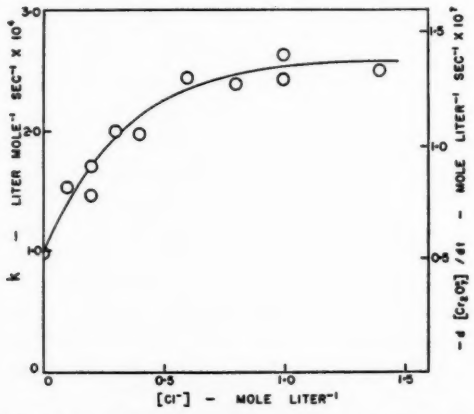


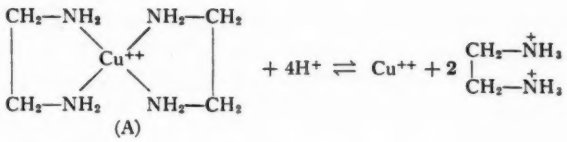
FIG. 5. Dependence of rate on Cl^- concentration. 0.10 *M* Cu^{++}, 20 atm. H_2, 110° C.

to attribute the measured value of k in this region to this complex.* This value (2.5×10^{-4} liter mole^{-1} sec.$^{-1}$) is about 2.6 times as great as for the uncomplexed Cu^{++} ion.

At constant Cl^- concentrations the rate was found to be directly proportional to the total Cu (II) concentration confirming that the catalytic activity is associated with the cupric species.

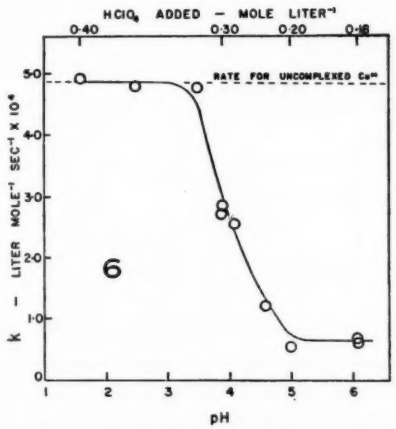
Cupric Ethylenediamine Complexes

The extent of complexing between Cu^{++} and EDA is a function of the H^+ concentration, i.e.



(A) [7]

The variation of k with pH, shown in Fig. 6, thus reflects a variation in the extent of complexing of the Cu^{++} ion.



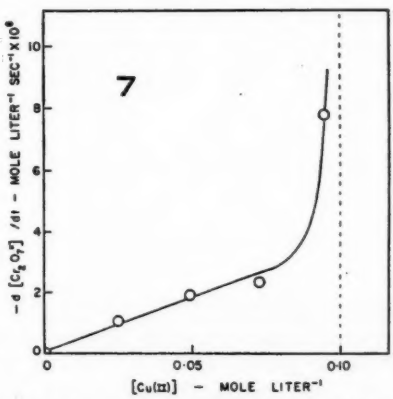


FIG. 6. Effect of pH on rate in cupric ethylenediamine solutions. 0.05 *M* Cu^{++}, 0.20 *M* EDA, 20 atm. H_2, 130° C. (pH measured at room temperature).

FIG. 7. Dependence of rate on cupric ion concentration in ethylenediamine solution. 0.20 *M* EDA, 20 atm. H_2, 130° C., pH 6 (measured at room temperature).

At low pH (<3, measured at room temperature) dissociation of the complex is apparently complete and the observed value of k approaches that for the uncomplexed Cu^{++} ion. Increasing the pH (by decreasing the amount of added $HClO_4$) results in a lowering of the rate, presumably reflecting the lower

**Spectrophotometrically determined values of the complexity constants of cupric chloride complexes at room temperature (8) suggest that higher Cl^- concentrations would be required to achieve complete complexing. However, it has been pointed out that these values probably increase with temperature (13).*

catalytic activity of the $Cu(EDA)_2^{++}$ complex (A). At high pH (>5) the rate levels off at a value ($k \approx 0.6 \times 10^{-4}$ liter mole^{-1} sec.$^{-1}$ at 130° C.), which is about one eighth that for the uncomplexed Cu^{++} ion at the same temperature. Room temperature values of the complexity constants (6) suggest that in this region complexing is essentially complete, all the Cu (II) being present as the $Cu(EDA)_2^{++}$ complex; hence it seems reasonable to associate the measured value of the k with this complex.

This interpretation is consistent with the results shown in Fig. 7. In a series of experiments in which the Cu (II) concentration was increased while the total EDA concentration was held constant, the rate (and the apparent value of k) rose sharply as the ratio [Cu (II)]: [EDA] approached the value 0.5.

Cupric Glycinate Complexes

Figs. 8 and 9 depict the effect of Gl on the rate of catalytic activation of H_2 by Cu^{++} in solutions of different pH.

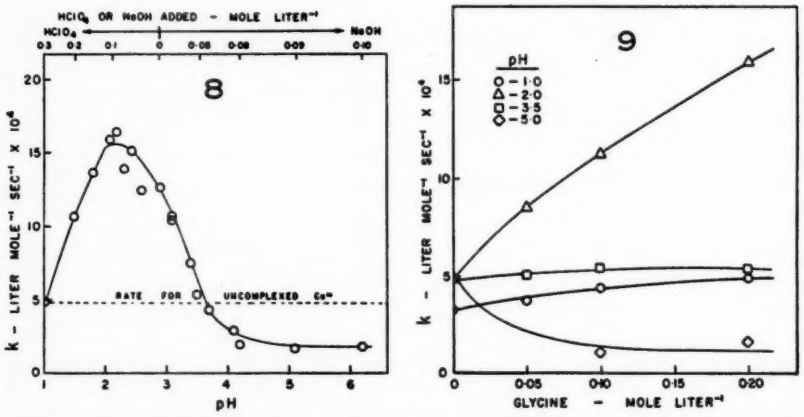
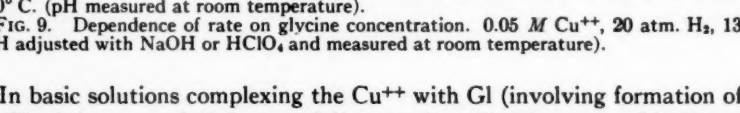


FIG. 8. Effect of pH on rate in cupric glycine solution. 0.05 *M* Cu^{++}, 0.20 *M* Gl, 20 atm. H_2, 130° C. (pH measured at room temperature).

FIG. 9. Dependence of rate on glycine concentration. 0.05 *M* Cu^{++}, 20 atm. H_2, 130° C. (pH adjusted with NaOH or $HClO_4$ and measured at room temperature).

In basic solutions complexing the Cu^{++} with Gl (involving formation of the $CuGl_2$ chelate complex) is essentially complete (6). However, with decreasing pH it is probable that the complex undergoes dissociation, passing through a number of intermediate stages including the following:



$$\rightleftharpoons Cu^{++} + 2HOC{-}CH_2{-}NH_3^+ \quad [8]$$

Consistent with this it was found that at low pH (≈ 1), the value of k approaches that for the undissociated Cu^{++} ion (Fig. 8) and is essentially independent of the Gl concentration (Fig. 9).

In the region of high pH (>3.5), k decreases with increasing pH (Fig. 8) and with increasing Gl concentration (Fig. 9) approaching a value ($\approx 1.5 \times 10^{-4}$ liter mole^{-1} sec.$^{-1}$ at 130° C.) about one third that of the uncomplexed Cu^{++} ion. This value, which presumably reflects the catalytic activity of the $CuGl_2$ complex (B), is subject to considerable uncertainty because of the competing side reactions between $Cr_2O_7^=$ and Gl (see Fig. 1).

In the region of intermediate pH (1–3.5) the measured value of k is greater than that for Cu^{++} and increases with the Gl concentration, presumably reflecting the presence of cupric carboxylate complexes such as (C). In common with the other cupric carboxylate complexes discussed earlier, the catalytic activity of this complex is apparently greater than that of the uncomplexed Cu^{++} ion.

CONCLUSIONS

The data obtained in this work have been used to compile Table II in which the various complexes, for which values of k have been determined, are arranged in order of decreasing catalytic activity. The listed values of the

TABLE II

RELATIVE CATALYTIC ACTIVITIES OF VARIOUS CUPRIC COMPLEXES

Complex	Catalytic activity*
$CuBu_2$	150
$CuPr_2$	150
$CuAc_2$	120
$CuSO_4$	6.5
$CuCl_4^=$	2.5
Cu^{++}	1
$CuGl_2$	<0.5
$Cu(EDA)_2^{++}$	0.1

*Relative to that of Cu^{++}.

relative catalytic activities (that of the uncomplexed Cu^{++} ion being taken as unity) are only approximate since they are based on rate measurements at different temperatures (ranging from 100° to 130° C.) and of varying precision. Of particular interest is the fact that negative ions (such as Ac^-, $SO_4^=$, Cl^-) enhance the catalytic activity of Cu^{++} while chelating reagents containing amine groups reduce it.

The similarity of the pattern of kinetic behavior for the different cupric complexes suggests that they all activate H_2 by a common mechanism. As proposed earlier (10, 11), the reduction of $Cr_2O_7^=$ may be depicted in each case as occurring through the following sequence of steps:

$$\text{Cu (II)} + H_2 \xrightarrow[\text{slow}]{k} X \qquad [9]$$

$$X + Cr_2O_7^= \xrightarrow[\text{fast}]{} \text{Cu (II)} + \text{products.} \qquad [10]$$

The detailed configuration of the active reducing intermediate (X) has not been definitely resolved, but it has been suggested (5) that the activation process may involve electron displacement from the H_2 molecule to the catalytic Cu (II) species. Support for this suggestion is provided by the fact that the effects of the various complexing agents on the catalytic activity, noted above, follow the same general pattern as has been observed for other reactions between metal ions (such as isotopic electron exchange) which are believed to occur by electron transfer mechanisms. Such reactions are commonly catalyzed by complexing anions (1, 3, 15).

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NOTES

UNIT CELL, SPACE GROUP, AND INDEXED X-RAY DIFFRACTION POWDER DATA FOR POTASSIUM HYDROGEN MALONATE, $KHC_3H_2O_4$[1]

By M. P. Gupta[2] and W. H. Barnes

The crystal structure of malonic acid has been the subject of two investigations (1, 2) but the configuration of the malonate group has not been determined in detail. In the hope that the introduction of a heavy atom may assist in the establishment of phase angles, a study of potassium hydrogen malonate has now been undertaken. Considerable delay, however, is anticipated before the work on the structure can be completed. The unit cell constants, space group, and X-ray diffraction powder data, therefore, are given in the present note as an aid to the identification of the acid salt, and the opportunity is taken of recording an interesting observation regarding the preparation of dipotassium malonate.

According to Groth (3), crystals of potassium hydrogen malonate, $KHC_3H_2O_4$, are monoclinic prismatic with $a:b:c = 1.1981:1:0.8121$, $\beta = 136°52'$, and show the forms {110}, {001}, {011}, {010}. In this laboratory the acid salt has been prepared by mixing aqueous solutions containing the appropriate stoichiometric proportions of malonic acid and potassium hydroxide, respectively, and allowing crystallization to proceed by evaporation of the solvent at room temperature. The crystals thus obtained have predominant forms corresponding to those designated {001} and {110} by Groth.

Zero-level precession photographs, taken with Mo K_α radiation ($\lambda = 0.7107$Å), along all three principal axes show that the diffraction symmetry is $2/mC-/-$. The unit cell constants are $a = 13.75$, $b = 11.61$, $c = 4.79$ Å, $\beta = 136°12'$. The axial ratio, $a:b:c = 1.184:1:0.4125$, is in reasonable agreement with that given by Groth if the latter's c/b ratio is divided by two. The density, measured at ~22°C. by flotation in mixtures of methylene iodide and toluene, is 1.81 gm./ml. There are, therefore, four molecules of $KHC_3H_2O_4$ in the unit cell; calculated density, 1.79 gm./ml.

Three space groups are compatible with the observed diffraction symmetry. Of these, $C2/m$, with eight general positions, may be eliminated from further consideration on the grounds that there are only four molecules of $KOOC.CH_2.COOH$ in the unit cell and each of these obviously must be asymmetric. Although no response was obtained from the crystals in a piezoelectric test, definite significance cannot be attached to this observation. An attempt was made to distinguish between the two other possible space groups $C2$ and Cm, on the basis of the statistical distribution of reflection intensities (4) in the $\{h0l\}$ zone. In the plot of $N(z)\%$ against $z\%$(2) the points at $z\% = 20$, 30, 40, 50, 90, 100 lie very close to the acentric curve, that at $z\% = 10$ is midway

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between the acentric and centric curves, while those at $z\% = 60, 70, 80$ agree better with the centric curve. These results are not completely free of ambiguity but they do strongly suggest an acentric distribution in the $\{h0l\}$ zone, and hence the space group Cm for the crystal structure. Wilson's ratio (5), $\rho = \langle|F|\rangle^2/\langle I\rangle = 0.716$, unfortunately is almost exactly the mean of the theoretical values for centric (0.637) and acentric (0.785) distributions. The forms observed on the crystals are too few for definite conclusions from the crystal morphology but the crystal class appears to be m, again suggesting Cm (rather than $C2$) as the space group.

A better choice of the unit cell (with $c < a < b$ and β closer to 90°) than that corresponding with Groth's orientation is obtained by application of the transformation matrix 102/010/001. The dimensions of the new cell are $a = 9.52$, $b = 11.61$, $c = 4.79$ Å, with $\beta = 92°6'$. There are four molecules in this unit cell, and the probable space group, as before, is Cm.

A powder photograph of potassium hydrogen malonate is reproduced in Fig. 1. It was obtained with Co K_α radiation ($\lambda = 1.790$Å) in a cylindrical

FIG. 1. X-ray diffraction powder photograph of potassium hydrogen malonate, $KHC_3H_2O_4$. (Camera diameter: 114.6 mm.; radiation: Co K_α, $\lambda = 1.790$Å.)

camera (114.6 cm. diameter), Straumanis film mounting, apparatus "cut-off" 20Å. Only the transmission section of the film is shown although the pattern extends almost to the limit of the back-reflection region. The measured spacings (d) and the visually-estimated relative intensities (I/I_1) of the lines of the powder pattern are recorded in Table I, where B identifies lines that were broader than the average, and an asterisk (in the column d(Obs.)) indicates the possible presence of a line the intensity of which was too low ($I/I_1 < 1$) for accurate determination of d. The lines of the pattern have been indexed for $d > 2.00$Å on the basis of the unit cell constants given in the preceding paragraph.

In case it should be observed in other laboratories, a very weak line of $d = 4.56$Å, which cannot be indexed, appeared in the powder photographs of two samples from the same preparation of potassium hydrogen malonate. Because this line is of significantly different relative intensity in the two films it was presumed to be due to the presence of an impurity; this was confirmed by its absence from a powder photograph of comparable density obtained from a second specimen of the salt. The spurious line cannot be due to potassium hydroxide, potassium carbonate, or malonic acid because the

powder patterns of none of these compounds have a strong line in the appropriate 2θ region. It was considered, therefore, that it might perhaps indicate the presence of a trace of the neutral salt.

TABLE I

X-RAY DIFFRACTION POWDER DATA (INDEXED FOR $d > 2$Å)

I/I_1	d(Å) Obs.	d(Å) Calc.	hkl	I/I_1	d(Å) Obs.	d(Å) Calc.	hkl	I/I_1	d(Å) Obs.	d(Å) Calc.	hkl
100	7.32	7.36	110	1	2.23	2.22	$24\bar{1}$	2	1.59		
30	5.78	5.81	020	—	—	2.21	$022, 33\bar{1}$	3	1.49		
	*	4.79	001	40	2.19	2.20	420	8	1.47		
—	—	4.76	200	1	2.18	2.18	241	10	1.44		
30	4.02	4.07	$11\bar{1}$			2.17	$20\bar{2}$	2	1.41		
40	3.92	3.96	111	1	2.15	2.16	$331, 40\bar{1}$	3B	1.39		
20	3.66	3.69	021	5	2.10	2.11	202	10	1.34		
		3.68	220			2.10	401	3	1.30		
20	3.56	3.58	130	—	—	2.05	$15\bar{1}$	1	1.26		
1	3.47	3.44	$20\bar{1}$	30	2.03	2.03	$151, 22\bar{2}$	1	1.25		
45	3.30	3.31	201				$42\bar{1}$	8B	1.23		
30	3.05	3.06	310	1	2.01	2.00	$13\bar{2}$	3	1.22		
1	2.98	2.96	$22\bar{1}$	8	1.97			1	1.21		
40	2.92	2.90	040	8	1.92			10	1.20		
75	2.88	2.89	$13\bar{1}$	2	1.90			3B	1.19		
		2.88	221	2	1.87			3	1.17		
3	2.83	2.85	131	8	1.85			3	1.16		
5	2.59	2.62	$31\bar{1}$	3	1.83			2	1.13		
1	2.52	2.54	311	1	1.78			2	1.12		
2	2.47	2.48	041, 240	10	1.76			2	1.10		
25	2.45	2.45	330	15B	1.72			2	1.09		
35	2.37	2.39	002	15	1.70			1	1.08		
		2.38	400	5	1.68			2	1.06		
1	2.31	2.30	$11\bar{2}$	10	1.65			2	1.05		
25	2.26	2.26	112, 150	15	1.63			5	1.02	($K_{\alpha 1}$)	

Two attempts were made to prepare dipotassium malonate by mixing solutions containing the base and the acid in the proportions of two moles of potassium hydroxide to one mole of malonic acid. In each case the resulting solution was boiled and set aside at room temperature. Both preparations yielded poorly-formed crystals from an oily, viscous liquid which had the usual appearance of a concentrated solution of potassium hydroxide. Both batches of crystals gave powder photographs identical with that obtained from potassium hydrogen malonate. Solutions of potassium hydroxide and malonic acid containing the base and the acid in the molar proportions of 3: 1 (i.e., 50% excess KOH) then were mixed and the solvent was evaporated by boiling until crystallization commenced. When cooled to room temperature the mixture had the same appearance as the previous preparations, but, upon stirring vigorously, almost instantaneous conversion to a solid mass occurred. The solid was brought back into solution by the addition of distilled water, and the volume was reduced by boiling until it was estimated that crystallization would not take place until the temperature was reduced almost to that of the room. No solid phase appeared, however, on cooling to about 22°C., but

crystallization was initiated by storage for a short time at 5°C. These crystals grew very rapidly at 22°C. into short, thick prisms of about 3 mm. cross-section, and the supernatant liquid was water-like in appearance. The crystals were reduced to powder and taken up in a capillary tube in a dry box because they proved to be somewhat deliquescent. The powder pattern of this material is not the same as that of the acid salt, nor does it have a strong line corresponding to $d = 4.56$Å.

The powder photograph of this new solid phase is not reproduced in the present note because, although it presumably represents that of dipotassium malonate, the composition of the crystals and the degree of possible hydration have not been determined. The large excess of KOH required for the formation of the neutral salt, and the tendency of the solution to supersaturation, suggest that a study of the reaction between potassium hydroxide and malonic acid, and a crystal structure determination of dipotassium malonate, might be of considerable interest. In order not to delay the investigation of the structure of potassium hydrogen malonate, however, the examination of the neutral salt has been discontinued for the present, and it has not been considered worth while to make further attempts to identify the impurity giving rise to the extraneous line of $d = 4.56$Å in the powder patterns of one of the two preparations of the acid salt.

Mr. B. J. Cowick assisted with the powder investigations.

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APPLICATION OF THE WISHAW–STOKES EQUATION TO THE CONDUCTANCES OF POTASSIUM CHLORIDE SOLUTIONS AT 25° C.

BY A. N. CAMPBELL AND L. ROSS

We have determined the conductances, densities, and viscosities of 2, 3, and 4 normal potassium chloride solutions; the latter concentration is almost the highest that can be attained at 25°. Campbell and Kartzmark (2) had previously calculated the conductances of potassium chloride solutions, using the equation of Wishaw and Stokes (5):

$$\Lambda = \left(\Lambda^\circ - \frac{B_2\sqrt{C}}{1+B\mathring{a}\sqrt{C}}\right)\left(1 - \frac{B_1\sqrt{C}.F}{1+B\mathring{a}\sqrt{C}}\right)\frac{\eta^\circ}{\eta}.$$

The meanings of the symbols in the above equation are given in the original paper. Campbell and Kartzmark obtained very good agreement between the observed and calculated conductances, up to a concentration of 1 normal, using the experimental values of the International Critical Tables, and an å value (distance of closest approach) of 4.6 Å.

In the present note, we have recalculated the results, using for Λ° the very accurate figure of Gunning and Gordon (3) (Λ° = 149.88 mhos) and using two different values of å, viz. 3.3 Å and 4.6 Å. The value 3.3 Å is recommended by Stokes himself (4) for dilute solutions, but we consider that the value 4.6 Å is just as good in the dilute region and reproduces the results for concentrated solutions much better than the lower value. In our recalculation, for dilute solutions, we have used the results of Gunning and Gordon (3) wherever they were applicable. Our technique has often been described. The complete results, both our own and the literature values, are contained in the table.

TABLE I

OBSERVED AND CALCULATED EQUIVALENT CONDUCTANCES OF KCl SOLUTIONS
t = 25.0° C.

C, moles per liter	Density, gm. per ml.	Relative viscosity (H_2O = 1.000)	Λ_{exp}, mhos	Λ_{calc}		α	
				å = 3.3	å = 4.6	å = 3.3	å = 4.6
0.0000	Viscosity and density as in literature		149.88	149.91	149.86	0.999	1.000
0.0005			147.79	147.82	147.78	0.999	1.000
0.0010			146.95	146.99	146.91	0.999	1.000
0.0020			145.79	145.86	145.90	0.999	0.999
0.0050			143.59	143.70	143.71	0.999	0.999
0.0100			141.30	141.41	141.68	0.999	0.997
0.0200			138.34	138.39	138.91	0.999	0.996
0.0500			133.37	133.09	134.19	1.002	0.994
0.1000			128.96	127.98	129.88	1.008	0.993
0.2000			123.90	121.95	125.00	1.016	0.991
0.5000			117.20	112.83	117.29	1.039	0.999
1.0000			111.90	105.30	111.96	1.063	0.999
2.0050	1.087	1.005	105.81	96.95	104.53	1.091	1.012
3.0000	1.130	1.030	100.38	89.99	98.04	1.115	1.024
3.9590	1.169	1.069	94.59	83.55	91.77	1.132	1.031

The table is almost self-explanatory. The values of $\alpha = \Lambda_{obs}/\Lambda_{calc}$ have no theoretical significance; they merely represent the discrepancy between observed and calculated results. The figures for observed and calculated conductances are plotted in the diagram (Fig. 1).

It is difficult to see why so "normal" a substance as potassium chloride fails to agree with the Wishaw–Stokes equation, as contrasted with the remarkable agreement for lithium nitrate (1). For potassium chloride the discrepancy amounts to 3% at 3.96 N (using å = 4.6 Å) and this is certainly much better than is given by any other conductance equation but we had hoped for something better. It may be that the weakness lies in using the bulk viscosity instead of some related function, the bulk viscosity being somewhat too great a dividing factor. We still think, however, that the Wishaw–Stokes extension of the Debye–Hückel equation is by far the most significant contribution to

the theory of electrolytic conductance that has been made since the inception of the Debye–Hückel theory.

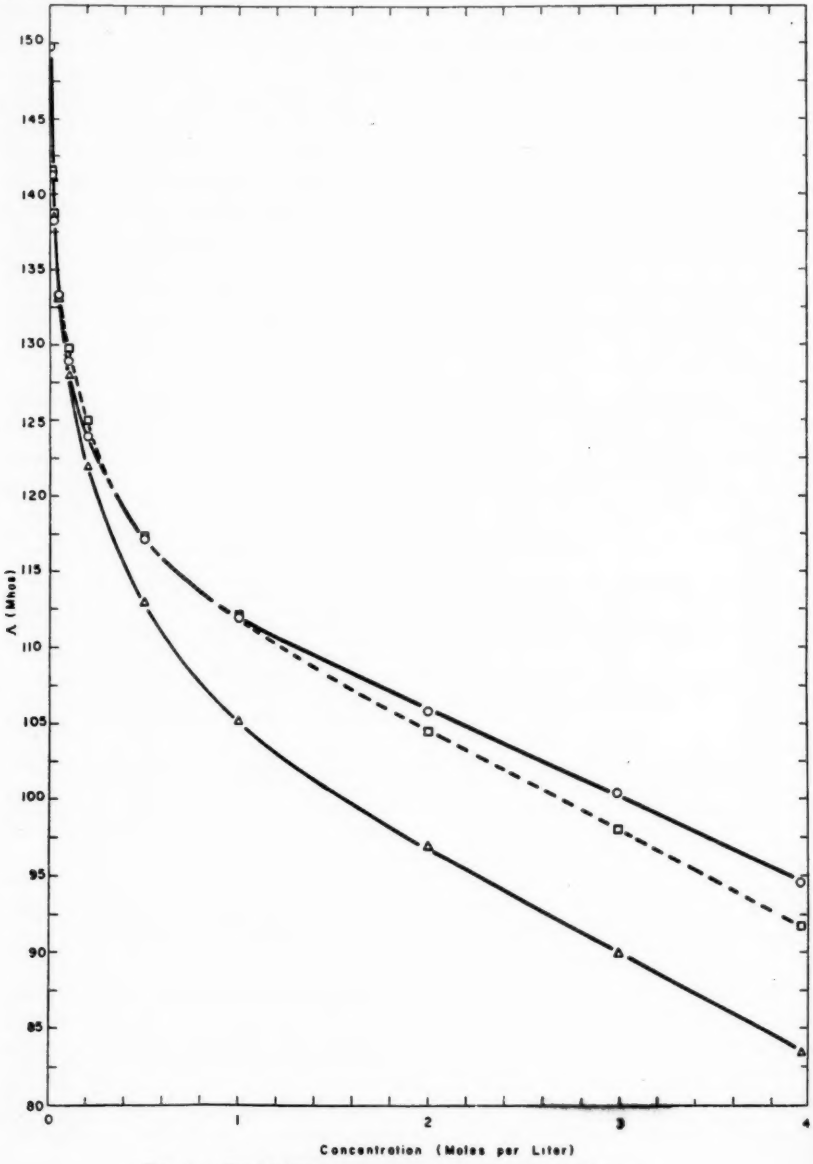

FIG. 1. Equivalent conductance versus concentration.
○ Experimental values for Λ.
△ Calculated values for Λ using å = 3.3.
□ Calculated values for Λ using å = 4.6.

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